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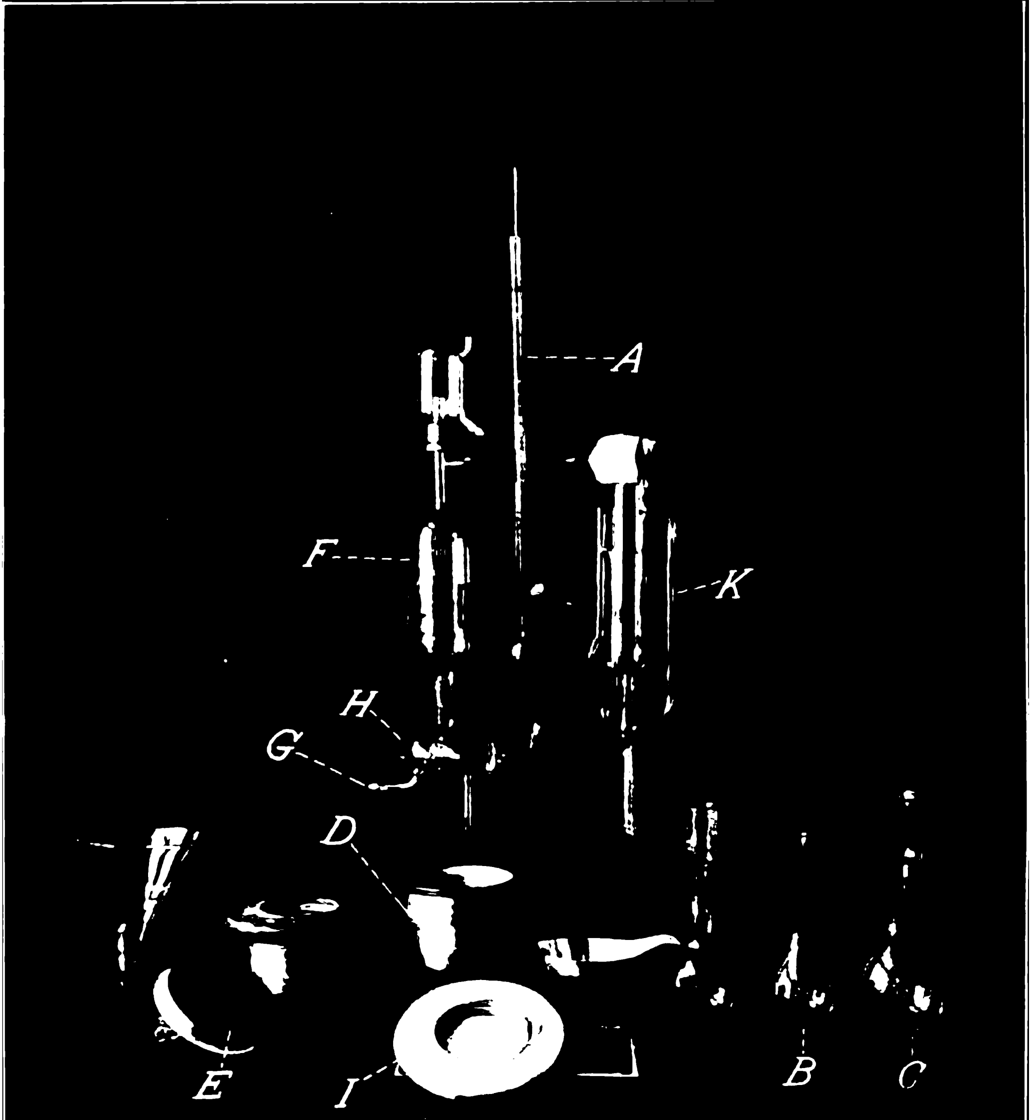

Bulletin

U.S. Bureau of Chemistry

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Issued August 4, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 143.

H. W. WILEY, Chief of Bureau.

THE FOOD LAWS OF THE UNITED KINGDOM AND THEIR ADMINISTRATION.

BY

F. L. DUNLAP,

Associate Chemist and Member of the Board of Food and Drug Inspection.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., May 4, 1911.

Sir: I have the honor to transmit for your approval a report on an investigation which I have made of the food laws of Great Britain and their administration. Due acknowledgment is made to Dr. G. S. Buchanan, Acting Chief Inspector of Foods of the Local Government Board, as well as to E. G. Haygarth Brown, Esq., Superintending Inspector, Board of Agriculture and Fisheries of Great Britain, for the aid which they have given me. I recommend that this report be published as Bulletin No. 143 of the Bureau of Chemistry.

Respectfully,

F. L. Dunlap,
Acting Chief.

Hon. James Wilson,
Secretary of Agriculture.

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THE FOOD LAWS OF THE UNITED KINGDOM AND THEIR ADMINISTRATION.

INTRODUCTION.

LIST OF ENGLISH FOOD LAWS.

The food and drugs act of the United States, enacted June 30, 1906, deals not only with foods and drugs which enter interstate commerce or are sold within the District of Columbia or the Territories, but also with those products which are either exported or imported. As far as exports are concerned the food law provides:

> (Sec. 2.) That no article shall be deemed misbranded or adulterated within the provisions of this act when intended for export to any foreign country and prepared or packed according to the specifications or directions of the foreign purchaser when no substance is used in the preparation or packing thereof in conflict with the laws of the foreign country to which said article is intended to be shipped * * *.

Food and drug imports coming into the United States are subject to examination on the part of the Secretary of Agriculture in order to determine whether or not they are adulterated or misbranded, and (sec. 11)—

> * * * if it appear from the examination of such samples that any article of food or drug offered to be imported into the United States is adulterated or misbranded within the meaning of this act, or is otherwise dangerous to the health of the people of the United States, or is of a kind forbidden entry into, or forbidden to be sold or restricted in sale in the country in which it is made or from which it is exported, or is otherwise falsely labeled in any respect, the said article shall be refused admission, and the Secretary of the Treasury shall refuse delivery to the consignee * * *.

If section 11 of our national law is to be strictly enforced a thorough working knowledge of the foreign food laws and their various regulations is necessary. Acquaintance with the methods of administration is also desirable. The problems are much the same in all countries where a serious attempt is made to combat the adulteration and misbranding of food and drugs; hence much of value may be learned by a study of the foreign food laws and their administration. Some of the laws which are in operation now in the United Kingdom governing the sale of foods date back to the early part of the eighteenth century. However, most of them, and also those covering different types of drugs, were passed much later. The following is a list of these laws now in force, with the dates of their passage:

1. Coffee act, 1718.
2. Coffee and tea act, 1724.
3. Coffee and tea act, 1730.
4. Tea act, 1776.
5. Bread act, 1836.
6. The sale of food and drugs act, 1875.
7. The sale of food and drugs act, 1879.
8. The margarine act, 1887.
9. The sale of food and drugs act, 1899.
10. Butter and margarine act, 1907.
11. Fertilizer and feeding stuffs act, 1906.
12. Public health (regulations as to food) act, 1907.
13. Merchandise marks acts, 1887–1894.

Nos. 6 to 10, inclusive, are known collectively as the sale of food and drugs acts, 1875–1907.

The most important of these acts now operative in England, Scotland, and Ireland, as far as foods and drugs are concerned, are the sale of food and drugs act of 1875 and its amendments, the margarine act of 1887, and its amended form as passed in the butter and margarine act of 1907.

DEFINITION OF TERMS.

In the sale of food and drugs act, 1899, the term "food" is defined as including "every article used for food or drink by man, other than drugs or water, and any article which ordinarily enters into or is used in the composition or preparation of human food; and shall also include flavoring matters and condiments." (The sale of food and drugs act, 1899, sec. 26.) This definition of "food" is in some senses more restricted than the definition in the United States food and drugs act, which, by use of the phrase "or other animals," includes those substances used as food by horses, swine, poultry, etc. In order to cover this field in Great Britain and Ireland, the fertilizers and feeding stuffs act of 1906 was enacted, and includes among the animals to which it applies cattle and poultry, "the term 'cattle,' for the purpose of this act, meaning 'bulls, cows, oxen, heifers, calves, sheep, goats, swine, and horses.'" (The fertilizers and feeding stuffs act, 1906, secs. 1 and 10.)

In the sale of food and drugs act, 1875, the term "food" was defined so as to "include every article used for food or drink by man other than drugs or water" (sec. 2).[1] In 1894 the English courts held that a baking powder composed of bicarbonate of soda, alum, and rice was not a food within the meaning of the act. In order to broaden the definition so as to include such substances as baking powder, the phraseology as already indicated was adopted in the act of 1899.

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[1] Note the similarity in the definition of "food" in the United States law: "include all articles used for food, drink, * * * by man * * *."

The food law of the United States follows, in certain particulars, quite closely the wording used in the act of 1875 in its definition of the word "food." In section 6 of the food and drugs act the term "food" is defined so as to include "all *articles used for food*, drink, confectionery, or condiment by man or other animals, whether simple, mixed, or compound." The italicized portion of this definition is identical with the wording of the act of 1875.

Under the Federal law all articles whether used per se or not for food are considered as coming within the above definition, thus including articles such as flour, baking powder, coal-tar dyes, etc. In this connection it is interesting to note the definition of misbranding as found in section 8, which provides "That the term 'misbranded,' as used herein, shall apply to all articles of * * * food, *or articles which enter into the composition of food*, * * * etc." No such phraseology as that italicized is found in the adulteration section, section 7.

In the sale of food and drugs act, 1875, the term "drug" includes "medicine for internal or external use" (sec. 2). This definition was not modified by the 1899 amendment. It is not so broad as the definition given in the United States law, according to which "all medicines and preparations recognized in the United States Pharmacopœia or National Formulary for internal or external use, and any substance or mixture of substances intended to be used for the cure, mitigation, or prevention of disease of either man or other animals," are subject to this act.

The same question has arisen under the English law as will doubtless arise in the enforcement of our own, namely, What is the status of those substances having a twofold function, first, that of a drug, and second, that of a substance used purely for technical purposes? In 1896 this question was decided in the English courts with reference to beeswax, a sample of which had been sold and on examination was discovered to contain about 50 per cent of paraffin. The sample was sold by a grocer and not by a druggist, and the courts held that although beeswax is used in a medicinal way and is recognized by the British Pharmacopœia, yet in the present case the product was not a drug. The point at issue seems to hinge on the question of whether or not the product sold is intended for medicinal purposes. A similar view has been taken by the United States Department of Agriculture. Substances often used as drugs, that is, recognized by the Pharmacopœia, but which are likewise used for technical purposes, if labeled "For technical purposes" and intended for such use, are considered as not subject to the food and drugs act. Food Inspection Decision No. 103, on "The labeling of turpentine," clearly sets forth the department's views respecting a substance recognized by the United States Pharmacopœia, yet having a large technical use as well. In this decision it is held that "when wood turpentine is labeled 'Not for medicinal use,' etc., it is not subject to the food and drugs act. When not so labeled it is in violation of section 7 of the food and drugs act unless labeled 'wood' or 'stump' turpentine."

This policy is outlined in Food Inspection Decision 58 and the same principle applies also to foods which have a technical use, as, for example, olive oil. With respect to importations this phase of the question is covered by Food Inspection Decision 93, which is an amendment to Regulation 34 of Circular 21, Revised, of the Secretary's office. This regulation as amended reads as follows:

> Unless otherwise declared on the invoice, all substances ordinarily used as food products will be treated as such. Shipments of substances ordinarily used as food products intended for technical purposes should be accompanied by a declaration stating that fact. Such products should be denatured before entry, but denaturing may be allowed under customs supervision, with the consent of the Secretary of the Treasury, or the Secretary of the Treasury may release such products without denaturing, under such conditions as may preclude the possibility of their use as food products.

ADMINISTRATIVE WORK AND METHODS OF ENFORCEMENT.

GENERAL ADMINISTRATIVE MEASURES.

Before entering into a further discussion of these laws it is desirable that a brief account of their method of enforcement be given. This control is very much subdivided and is somewhat complex. In some ways this subdivision of control and authority suggests the condition existing in the United States, although its existence is for different reasons. In the United States each State has its own separate law and its own controlling officers, while the Federal law controls only the manufacture and sale of foods and drugs in the District of Columbia and the Territories, the shipment of these commodities, in interstate and foreign commerce, and their sale in the "original package,"[1] although the line of demarcation between State and National control is sharply drawn. In the United States most of the States have laws modeled quite closely after the national law, while in the United Kingdom one and the same law is operative throughout Great Britain and Ireland. The sale of food and drugs acts specify the local authorities by whom the acts are to be administered (the county councils and certain town and borough councils), and they require every such authority to appoint a public analyst and to make arrangements for the collection of samples of foods to be examined by the public analyst. The appointments of public analysts have to be sanctioned by the Local Government Board, and a general supervision is exercised by that department over the methods of administration by the local authorities. Subject to such general control, it rests with each local authority, on the reports of the public analyst and other officers, to enforce the law against offenders, to issue warnings to traders, or take other action for the purpose of checking these forms of adulteration or misbranding of foods to which the sale of food and drugs acts are applicable. There are at present 233 local authorities administering the sale of food and drugs acts in England and Wales. These acts are the same in the jurisdiction of all local authorities and there are no special additions in local areas.[1]

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[1] The term "original package" here used means the original package as defined by the Supreme Court with respect to articles of interstate commerce. Decisions bearing on this point are found in Food Inspection Decision 86.

The Supreme Court decided March 13, 1911 (Hipolite Egg Co. *v.* U. S.), that adulterated and misbranded articles of food which have been transported in interstate commerce are liable to seizure within the boundaries of a State as long as they remain in the original unbroken packages.

The sale of food and drugs act, 1899, section 2, gives direct power to the Local Government Board and the Board of Agriculture and Fisheries to sample articles of food. By so doing these central authorities can determine whether or not the local authorities are doing their duty properly. The fees for the analyses are paid by the local authority where the samples are collected, and it is the duty of the local authority to cause proceedings to be instituted when the board makes adverse reports based on such analyses.

The sale of food and drugs act of 1875 was designed chiefly as a measure of administration, and made little provision for any direct action by the central departments of government, but the subsequent acts have provided for central action in several directions. There are now six government departments which in different ways are concerned in the administration of these laws, namely, the Local Government Board of England, and the Local Government Boards for Scotland and Ireland; the Board of Agriculture and Fisheries, which has jurisdiction in England and Scotland; the Department of Agriculture and Technical Instruction in Ireland, and the commissioners of customs and excise. These departments communicate with the local authorities on the matters with which they are concerned and when necessary cooperate with one another.

The act of 1899 gave special duties and authority to the Board of Agriculture and Fisheries as far as adulteration of agricultural products (butter, milk, cheese, etc.) was concerned, in addition to the general power of supervision in connection with the adulteration of all articles of food which is imposed on the Local Government Board in the interests of the consumer.

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[1] In this respect these sales of food and drugs acts differ from the public health acts. The public health acts make certain provisions as to unsound food, milk, etc., for the whole country, but in addition some local authorities have obtained clauses in special acts of Parliament giving them wider powers in their jurisdictions. Many local authorities have in this way special powers to secure the cleanliness of ice cream, while some of the principal towns have special "milk clauses" under which, when milk is found to contain tubercle bacilli, the milk from the herd giving the infected product may be prohibited entry into the town.

TECHNICAL QUESTIONS REFERRED TO SPECIAL COMMISSIONS.

MISCELLANEOUS REPORTS MADE BY MEDICAL DEPARTMENT, LOCAL GOVERNMENT BOARD.

An expert subdivision of the medical department of the Local Government Board has been established for five years, and handles technical and administrative questions relating to the purity and wholesomeness of the food and drug supply of the country. The duties required of this separate division are "to advise the board as to the administration of the sale of food and drugs acts and other acts relating to food questions; to deal with matters relating to the purity and adulteration of foods which are brought to the board's attention by public analysts, medical officers of health, and others; to obtain information upon special questions relating to the purity and adulteration of food, and the use of deleterious substances therein; and to make suitable inquiries and investigations for the purpose." At the present time the work is under the control of a chief inspector of foods and five inspectors.[1] The technical assistance necessary to carry on the work of this staff is obtained from various sources "by means of the allowance allowed to the subdepartment for laboratory assistance and investigation."

Beginning in 1906, the medical department of the Local Government Board has published from time to time special reports of the inspectors of foods, in addition to annual reports by the chief inspector. Up to the present time 13 reports have appeared. These reports are of great interest and of particular value in many ways to those who are interested in the relation of food to public health. Some of these reports likewise deal with other subjects of moment to the manufacturer of foodstuffs as well as to those who are interested in the administration of our various food laws, both State and National. The reports,[2] as far as published, are as follows:

1. On the changes in certain meat essences kept for several years in tins (1906). Dr. G. S. Buchanan and Dr. S. B. Schryver, D. Sc.
2. Lead and arsenic in tartaric acid, citric acid, and cream of tartar (1907). Dr. A. W. J. MacFadden.
3. On certain imported meat foods of questionable wholesomeness (1908). Dr. G. S. Buchanan.
4. On inquiries with regard to the wholesomeness of tripe of home and foreign origin (1908). Dr. A. W. J. MacFadden.
5. On the preparation and sale of vinegar in relation to the administration of the sale of food and drugs act (1908). Dr. J. M. Hamill.
6. On preservatives in meat foods packed in cans or glass (1908). Dr. A. W. J. MacFadden.

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[1] The acting chief inspector of foods is Dr. G. S. Buchanan.

[2] These reports are sold at from 3 to 4 pence apiece and may be had from Wyman & Sons (Ltd.), Fetter Lane, London, E. C.; Oliver & Boyd, Edinburgh; or E. Ponsonby, 116 Grafton Street, Dublin.

7. On the presence of tin in certain canned foods (1908). Dr. G. S. Buchanan and Dr. S. B. Schryver, D. Sc.

8. On "facing" and other methods of preparing rice for sale (1909). Dr. J. M. Hamill.

9. On the application of formaldehyde to meat (1909). Dr. G. S. Buchanan and Dr. S. B. Schryver, D. Sc.

10. On the use of preservatives in cream (1909). Dr. J. M. Hamill.

11. On a parasitic condition met with in Australian beef (1911). Dr. A. W. J. MacFadden and R. T. Leiper.

12. (a) On the bleaching of flour and the addition of so-called "improvers" to flour (1911). Dr. J. M. Hamill.

(b) On the chemical changes produced in flour by bleaching (1911). Dr. G. W. Monier-Williams.

13. On the presence of calcium sulphate in baking powder and self-raising flour (1911). Dr. J. M. Hamill.

As has been pointed out, the Local Government Board exercises a directing hand in the administrative work of the sale of food and drugs act as applied by the counties, boroughs, etc. When it appears that through laxity of method, or otherwise, satisfactory administration is not being obtained, the board recommends that the local authorities make such changes as must result in a more satisfactory state of affairs.

The reports of the inspectors of foods appear to have been of great value in aiding the proper and forceful administration of the law. With few exceptions, the food laws of Great Britain have no standards incorporated in the law itself or published otherwise for the guidance of manufacturers or the local authorities and analysts. In these reports of the inspectors suggestions for standards have been made, which appear to be of considerable value and assistance. For example, in Dr. MacFadden's report on "Lead and arsenic in tartaric acid, citric acid, and cream of tartar," a standard is suggested which limits the lead and arsenic[1] to a limit, respectively, of one-seventh and one one-hundredth grain in 1 pound of these substances. The lead figure was based on a "trade standard" which had been used for cream of tartar for some time, while the arsenic standard was based on the Final Report of the Royal Commission on Arsenical Poisoning (1903). Again, Dr. Hamill, in his report "on the preparation and sale of vinegar, in relation to the administration of the sale of food and drugs acts," discusses the fraud perpetrated on the consuming public because of an incorrect description in connection with the sale of various types of this food product, and states that if proper administrative control could be given to definitions similar to those found in the United States[2] it would operate to the advantage of the consumer and dealer.[3]

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[1] Calculated as arsenious oxid.

[2] Circular 19, Office of the Secretary, United States Department of Agriculture, "Standards of purity for food products."

[3] Report on work of the inspectors of foods for 1906-1908. A report by G. S. Buchanan, M. D., B. Sc. (1909).

COMMITTEE REPORT ON PRESERVATIVES AND COLORS.

From the administrative viewpoint, considerable attention has been paid in the United Kingdom to the propriety of the use of preservatives in foodstuffs. In order that this matter might be given a thorough investigation, the whole question was submitted to a committee, a very full report of which was published in 1901 under the title "Report of the departmental committee appointed to inquire into the use of preservatives and coloring matters in the preservation and coloring of food." This committee consisted of four members, and, with the exception of a minority report "Concerning the use of copper sulphate in the greening of vegetables," by Dr. F. W. Tunnicliffe, the recommendations of the committee were unanimous. Their recommendations are as follows:

(*a*) That the use of formaldehyde or formalin, or preparations thereof, in foods or drinks be absolutely prohibited, and that salicylic acid be not used in a greater proportion than 1 grain per pint in liquid food and 1 grain per pound in solid food, its presence in all cases to be declared.

(*b*) That the use of any preservative or coloring matter whatever in milk offered for sale in the United Kingdom be constituted an offense under the sale of food and drugs acts.

(*c*) That the only preservative which it shall be lawful to use in cream be boric acid or mixtures of boric acid and borax, and in amount not exceeding 0.25 per cent expressed as boric acid, the amount of such preservative to be notified by a label upon the vessel.

(*d*) That the only preservative permitted to be used in butter and margarine be boric acid or mixtures of boric acid and borax, to be used in proportions not exceeding 0.5 per cent expressed as boric acid.

(*e*) That in the case of all dietetic preparations intended for the use of invalids or infants chemical preservatives of all kinds be prohibited.

(*f*) That the use of copper salts in the so-called greening of preserved foods be prohibited.

These recommendations have never been enacted into law. Some of them are, however, used as a basis for prosecution in the United Kingdom under the sale of food and drugs acts, particularly in the case of milk to which preservatives have been added.[1]

With reference to the above recommendations it should be pointed out that Dr. F. W. Tunnicliffe put in a minority report on "The use of copper sulphate in the greening of preserved vegetables, etc." This report is as follows:

I agree with the above report, except as to paragraph 127 and recommendation F. With regard to the question of the addition of copper sulphate to preserved vegetables and fruits for the purpose of rendering them permanently green I regret that I am not quite in agreement with my colleagues. I regard it as established that these substances, as well as many other articles of diet, naturally contain copper, and that copper is constantly being introduced into food by the ordinary culinary processes, and further that although the copper is added in a soluble and absorbable form to the vegetables, it is not so present in them as consumed, being converted by them into a relatively insoluble and unabsorbable compound. I can conceive of no conditions under which the small quantity of copper present in the above form in properly preserved peas could be injurious to any consumer to whom the peas themselves would be harmless. In addition, I can see, so far as concerns a possible injurious effect, no analogy between this compound of copper in green vegetables which are eaten by the ounce and a highly soluble salt of lead in water, or of arsenic in beer, both liquids drunk by the quart or gallon. It must be remembered also in this connection that in France an order was issued prohibiting the use of copper for the above purpose and that this order had subsequently to be rescinded. It also appears that in Germany, where the use of copper for artificial greening of vegetables, etc., is prohibited, preserved vegetables containing copper are easily obtainable on the open market, apparently showing that the actual enforcement of the prohibition is attended with difficulty.

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[1] The Local Government Board has power to fix standards on preservatives in butter and margarine under section 7 of the butter and margarine act.

Recent research has distinctly taught us that, from the point of view of its nutritive value, great importance attaches to the appetizing appearance of food, and in my opinion we should not without very definite reason arbitrarily prevent the gratification of the public taste for a perennial supply of green vegetables and thereby destroy if not an important at least a thriving industry.

I am, however, satisfied that often an unnecessarily large amount of copper is present in vegetables permanently colored by means of it, and although in spite of diligent inquiry no injurious results have been known to have accrued even from these quantities, yet nevertheless only the necessary amount should be added. I should, therefore, recommend that the presence of copper in these preserved vegetables be in every case declared and that its amount be restricted to half a grain of metallic copper per pound.

Paragraph 127 referred to by Mr. Tunnicliffe is as follows:

127. The employment of copper sulphate to color peas and other vegetables has been carefully considered by us. It is highly undesirable that what is admittedly a poisonous drug should be used, even to the smallest extent, in connection with such food as may be consumed in considerable quantity. The public have got into their heads that vegetables ought to be green, and green they insist upon having them. Direct proof that vegetables containing copper are injurious to the consumer is from the very nature of the case difficult to obtain, and we must admit that we have not succeeded in obtaining it. There is evidence pointing to the conclusion that the copper, when added to the vegetables, forms a compound which is not easily soluble in the human economy. There is, however, evidence of a contrary character, and it is not clear to us that the whole of the copper added becomes, or remains, insoluble under all conditions. Be this as it may, recent events have so incontestably demonstrated the serious and widespread mischief which may result from the consumption of food and drink, other than sweetmeats, containing even minimal quantities of poisonous metallic substances, that we are strongly of opinion that such poisonous substances should be rigorously excluded.

It might be pointed out that the recommendations of this committee are not at one with the views which have found expression in the administration of the food law of the United States. Salicylic acid is looked upon as being of a nature requiring absolute prohibition in foodstuffs; the same is true of boric acid and borax; and the question as to the propriety of the use of copper salts in the greening of vegetables is at the present time in the hands of the referee board of consulting scientific experts, who are expected to report to the

Secretary of Agriculture as to whether they should be used for the purpose indicated.

COMMISSION REPORT ON WHISKY AND OTHER POTABLE SPIRITS.

In the United Kingdom the subject of whisky and other potable spirits has also been submitted to a commission, and only recently (1909) it reported the results of its investigation under the title of "The final report of the Royal commission on whisky and other potable spirits." The Royal commission definition of "What is whisky" is fundamentally the same as that of President Taft, namely, that, broadly speaking, all distillates from grain, if of potable strength, are "whisky."

There is an evident tendency to submit the more important questions arising in the administration of the sale of food and drugs acts to a commission for the taking of testimony and for the preparation of the reports to be used for the guidance of those who must administer these laws.[1] The report of the Royal commission on whisky and other potable spirits is, however, the second report on this subject. The first was issued about 20 years ago, and is entitled "Report from the Select Committee on British and Foreign Spirits" (1890–91).

CONTROL OF IMPORTS BY THE COMMISSIONERS OF CUSTOMS.

SCOPE OF WORK COMPARED WITH THAT OF LOCAL GOVERNMENT BOARD.

It has been pointed out that the commissioners of customs operate under the sale of food and drugs act at the ports of entry. This duty was laid upon them in the act of 1899. As an administrative detail of this work of the commissioners of customs it is to be noted that it is the commissioners themselves who must enforce the provisions and not the local authorities as in the case of the general provisions of this act. As far as their authority extends it is broad enough to control all types of foods offered for import, although particular provisions are made covering dairy products or substitutes for dairy products, as margarine, adulterated butter, condensed milk, etc. His Majesty may, by order in council, direct this section (11) of the sale of food and drugs act, 1899, to apply to "any adulterated or impoverished article of food," but a study of the work of the commissioners shows that its duties are more closely confined to the specific provisions of this section applying to dairy products and substitutes therefor. However, any adulterated or impoverished article of food, if plainly labeled to indicate the fact that it has been so treated, is not subject to these provisions.

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[1] In the address of the president of the Society of Public Analysts and of other Analytical Chemists, published in the February number of the Analyst, 1911, reference is made to a conference organized by the County Councils' Association which was held in London in March, 1910. At this conference it was recommended that "an expert board of reference should be constituted to decide controversial questions of chemistry, hygiene, or physiology." This conference also passed a resolution with regard to the fertilizers and feeding stuffs acts that "it was a matter of urgency that the question of 'standards' under the fertilizers and feeding stuffs acts should be dealt with by an expert board of reference."

The analyses of samples taken by the commissioners of customs are always made by the Government chemists at the bureau of inland revenue, and in this way also their work is differentiated from the work of the local authorities, who use the local analysts, except under conditions which will be outlined later.

In the annual report of the Local Government Board for 1909–10 it is stated that there are 233 districts for which this board has approved of the appointment of analysts for the purpose of making examinations under the sale of food and drugs act. These analysts examined during the course of the year 1909 a total of 97,985 samples collected in England and Wales. In 1908, 95,664 samples were collected, 8,169 were reported adversely, legal proceedings were begun in 3,643 cases, and penalties imposed by the courts in 2,673 cases. During 1908 there were 8,827 samples collected in Scotland and 9,694 in 1909.

It is pointed out in the annual report of the Local Government Board for 1907–8 "that these trifling fines, against which legal authorities have often protested, are useless for the purpose of repressing adulterations." In the United States, in addition to the fines which may be imposed, there is provided by law an effective deterrent, namely, the publication by the Government of notices of judgments of the courts; and liability to imprisonment on conviction for second and subsequent offenses.

It is almost needless to say that the results given indicate that the sale of food and drugs acts have accomplished and are accomplishing splendid work in Great Britain and are being ably administered. One noticeable feature is the fact that many of the cases are closely contested in the courts, while in the United States, under the Federal law this occurs less often. In order, however, to make such a comparison satisfactory the results obtained under the various State laws would have to be included.

TEA, DRUGS, AND OTHER PRODUCTS.

Three thousand two hundred and eighty-nine drugs were examined during the year 1908. Of this number 287, or 9 per cent, were found to be adulterated. The largest number of examinations were of camphorated oil (504 samples), cream of tartar (245 samples), and powdered licorice (184 samples).

While the greatest activity, as in previous years, was confined to the examination of dairy products and their substitutes, yet a considerable number of other food products were examined by the public analysts, including baking powder, fish, honey, olive oils, spices, sirup and treacle, wines (nonalcoholic), yeast, mustard, coffee, tea, vinegar, ginger, ice cream, canned meats, oat meal, rice, etc.

The commissioners of customs examined during the year ending December 31, 1908, under section 30 of the sale of food and drugs act, 1875, 4,347 samples of tea, of which 3,840 were satisfactory.[1] During the year 1909, 7,647 samples of the tea were examined, of which 7,089 were considered satisfactory. The tea which examination shows to be unsatisfactory is either exported or, sometimes, allowed entry as the raw product for the manufacture of caffein.

In the United Kingdom the importation of tea is controlled by the customs officials, and they decide whether or not the importations are fit for entry. The control of tea importations into the United Kingdom is under section 30 of the sale of food and drugs act, 1875, and aims at preventing the entry of all tea unfit for food. In the United States tea dust is allowed entry for the manufacture of caffein under a specific act of Congress.[2]

As an interesting comparison, and in order to indicate the extent to which inspection at ports in the United States has developed under the food and drugs act, the following quotation from the Report of the Secretary of Agriculture for 1909 (p. 35) will suffice:

> Of imported products the branch laboratories examined 8,476 samples, about 2,500 of which were sent to Washington for reexamination. In addition, more than 79,000 samples of imported goods were submitted to floor inspection at ports of entry, without examination in the laboratory.

DAIRY PRODUCTS.

With respect to the control of dairy products, butter, condensed milk, fresh milk, cream, margarine cheese, and margarine at the ports, His Majesty's customs operating under section 1 of the sale of food and drugs act, 1899, and section 5 of the butter and margarine act, 1907, collected and examined during the year ending December 31, 1908, 1,920 samples, of which 1,169 were butter and 120 samples condensed milk, including dried milk, and of this number 3 had been called "machine skimmed milk" or "skimmed milk" as required by section 1 (1) (*c*) of the sale of food and drugs act, 1899. Five hundred and eight samples of margarine were drawn and 11 found to contain over the legal amount of 16 per cent of water. During the year ending December 31, 1909, the officers of the board of customs and excise at the ports in the United Kingdom, with a view to giving effect to section 1 of the sale of food and drugs act, 1899, and section 5 of the butter and margarine act, 1907, collected the following number of samples for examination: Butter, 1,111; condensed and dried milk, 125; fresh and sterilized milk, 10; cream, 73; margarine, 510; total, 1,829.

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[1] To illustrate the extent of the tea inspection at the ports, for the year ending Mar. 1, 1908, 3,952 samples of tea were examined at the ports, representing an importation of 317,065,320 pounds.

[2] Regulation of importation of teas, Rev. Stat., sec. 7698–7707, U. S. Stat. L. (1907–1909), p. 163 (chap. 170).

While these are specific laws on our statute books controlling the importation of tea, yet the Attorney General has rendered an opinion in which he holds that these acts governing the importation of tea must be read as one with the food and drugs act, June 30, 1906.

Quite recently cooperation between the Treasury Department and the Department of Agriculture has been established looking to the application of the food law to importations of tea.

The chief work of the customs authorities under the sale of food and drugs acts relates to certain prescribed articles of dairy produce for which special provision has been made in the law, and to the examination of tea. In addition, however, an extensive system of inspection of foods at the ports of entry has now been established by regulation made by the Local Government Board under the public health (regulations as to food) act, 1907. In connection with this inspection, foods may be sampled and analyzed independently of the sale of food and drugs acts. The administration of these regulations rests primarily with the port sanitary authorities.

GENERAL SANITARY INSPECTION.

Much is done in general sanitary inspection, and the value of the products examined is enormous. In an article on "Food inspection at the ports of entry" in the Journal of the Royal Sanitary Institute, volume 29, page 681 (1908-9), W. F. Dearden, medical health officer at the port of Manchester, says: "The value of foreign foods inspected during 1906 was, in round numbers, assessed at £200,000,000, and that this amount has not reached finality is shown by the fact that in five years the value has increased by about £30,000,000."

At this point it should be stated that the public health (regulation as to food) act, 1907, includes "the power of making regulations authorizing measures to be taken for the prevention of danger arising to public health from the importation, preparation, storage, and distribution of articles of food or drink, other than drugs or water, intended for sale or human consumption."

The United Kingdom must depend largely for its butter supply on importations. More or less adulterated butter is met with at the ports, and an effort is being made to remedy this state of affairs. Early in the year 1906 a conference was held at The Hague on the subject, which was attended by the principal chemist of the Government laboratories and one of the superintending inspectors of the Board of Agriculture and Fisheries, who conferred with officials of the Netherlands Government. The proper inspection of factories seems to be desirable, and this point is brought out in the reports discussing the work of the customs, as follows:

> Hitherto under the law of the Netherlands the Dutch inspectors have had power to enter creameries, butter factories, and shops and warehouses where butter is stored or sold, but they have no power of entry into margarine factories.[1] Consequently it

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[1] This defect has now been remedied by recent legislation of the Netherlands Government.

has been the practice of persons engaged in the adulteration of butter to call their premises margarine factories.

In the United Kingdom, inspectors have had power to enter margarine factories, but no corresponding power as regards butter factories.[1] Consequently persons engaged in adding foreign fats to butter in this country have described their premises as butter factories.[2]

One of the difficulties under which the customs authorities labor is the lack of power to detain shipments pending the return of the results of analyses. By the time the analytical results are received by the customs officials the shipments are ordinarily all distributed and have gone into consumption. It was also the practice for the Board of Agriculture and Fisheries to communicate to the consignees and to the local authorities of the district in which the consignee lived that samples had been taken by the customs officials. This practice has been abandoned, as it did not yield satisfactory results.

LABELING OF IMPORTS UNDER THE MERCHANDISE MARKS ACTS, 1887–1891.

Under the merchandise marks acts, 1887–1891, the customs authorities have the opportunity to control the labeling of imported goods, including foods and drugs. Briefly summarized, their powers are limited to the detention of:

1. Any imported goods bearing marks or descriptions which are misleading as to the character, composition, or origin of the goods so marked or described, and,
2. Any imported goods of foreign manufacture bearing a name or trade-mark which is, or purports to be, the name or trade-mark of any manufacturer of, or dealer in, goods of the same description in the United Kingdom, and is unaccompanied by a definite indication of the country of origin of the goods.[3]

That the merchandise marks acts can be made to serve a very useful purpose so far as the labeling of foods is concerned is evident from the fact that, for example, consignments of spirits, distilled in European countries, in bottles labeled "finest old Scotch whisky," "vieux Cognac," "fine champagne," and "fine old Jamaica rum," were seized.

In this connection it is of interest to note the attitude taken by the customs authorities with respect to the labeling of port and sherry, as indicated by a general order issued on January 1, 1906:

> Entries in which wine from countries other than Portugal is described as "port," unaccompanied by satisfactory evidence that the wine is the product of that country, should not be accepted unless the word "port" is qualified by an unmistakable indication of the country in which the wine was produced, such as Spanish port, French port, or German port. Such descriptions as Tarragona port, Catalonia port, Roussillon

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[1] This defect has been remedied by the butter and margarine act, 1907.

[2] Annual Report of Proceedings under the Sale of Food and Drugs Acts, 1875–1899, etc., for the year 1906.

[3] Fiftieth Annual Report of Commissioner of His Majesty's Customs for the year ending 31st of March, 1906, p. 33.

port, or Hamburg port, must be accompanied by the words "produce of Spain," "produce of France," or "produce of Germany," as the case may be. Wines described as sherry imported from countries other than Spain should be similarly qualified."[1]

The same principle is found in Food Inspection Decision No. 122, wherein it is stated that the ports and sherries produced in California must be labeled respectively "California port," "California sherry."

EXTENT OF INSPECTION AND SAMPLING UNDER THE VARIOUS LAWS.

SPECIAL EXAMINATION OF DAIRY PRODUCTS.

That dairy products receive a great amount of attention in the United Kingdom is shown by the fact that during the year 1908, 45,093 samples of milk were examined, of which 10.5 per cent were reported as either adulterated or as falling below the minimal limits fixed by the "sale of milk regulations, 1901." These regulations do not fix a minimum percentage. Under the regulations a presumption of adulteration or abstraction is raised by a deficiency of milk fat or other solids, but if it can be proved that the milk was sold as it came from the cow, this presumption is rebutted. The sale of milk containing less than 3 per cent of milk fat, or less than 8.5 per cent of other milk solids, does not therefore necessarily constitute an offense. The effect of the regulations is merely that proof of the deficiency throws on the defendant the onus of showing by direct and positive evidence that the milk was sold as it came from the cow. The merely "presumptive" nature of these limits has given rise to a considerable agitation by local authorities for legislation establishing absolute limits.

At this point for purposes of comparison it might be pointed out that in the standards fixed in Circular No. 19 of the Office of the Secretary of Agriculture, the standard for butter fat in milk is set at 3.25 per cent. Even this is low, a milk of good average quality carrying more nearly 3.5 per cent or more. The examinations of the public analysts have resulted in the cases being brought primarily on the addition of water to milk, the abstraction of its fat, and the addition of preservatives. It is, however, gratifying to learn that drastic action, usually under other acts, has been taken for the sale of dirty milk. In one case (in 1907) in Westminster a dealer was "dealt with under the public health act, 1875, and sent to prison for six months." During the year 1909 the number of milk samples taken in England and Wales under the sale of food and drugs acts were 45,576 and of butter 20,670. During the same year in Scotland the samples of milk and butter examined were respectively 4,833 and 1,838. Of the 20,729 butter samples examined in 1908, 1,545, or 7.5 per cent, were reported adversely; 1,273 of these samples contained foreign fat.

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[1] Fiftieth Annual Report of Commissioner of His Majesty's Customs for the year ending 31st of March, 1906, p. 35.

Under the sale of food and drugs act, 1899, and the butter and margarine act, 1907, the maximum limit of butter that may be mixed with margarine is 10 per cent. In 1907, 31 samples of margarine were collected from one place in Birmingham. Of these, 12 contained from 38 to 83 per cent of butter.

INSPECTION UNDER THE BUTTER AND MARGARINE ACT, 1907.

The butter and margarine act, 1907, which is really part of the sale of food and drugs acts, 1899, is administered by the Board of Agriculture and Fisheries. This act became operative on January 1, 1908, and at present there are three inspectors (not including the local inspectors appointed by the town and borough councils) at work in Great Britain to carry it into effect. These inspectors collect samples of butter and margarine, together with a few samples of mixtures of milk and butter. During the year 1910 a total of 459 samples were taken. The Board of Agriculture and Fisheries also cooperates with the local inspectors who in some districts take samples under this act. The samples collected under the act are sent to the Bureau of Inland Revenue in London for analysis.

In Ireland there are 4 regular inspectors who look after the butter factories under the butter and margarine act, 1907, and 2 inspectors who take samples from shops. Besides these, there are 10 dairy instructors under the control of the Department of Agriculture and Technical Instruction for Ireland, and these 10 have the power of sampling. There are also 34 county instructors in dairying who are under the supervision of the department, and it is probable that the authority to sample will be extended to them also.

SAMPLING UNDER THE SALE OF FOOD AND DRUGS ACT.

While His Majesty's customs, as far as the inspection force is concerned, confine their attention to agricultural products (see p. 18), it is found that analyses of these products by the local authorities within the country is much more extensive.

For the year ending December 31, 1907,[1] the total number of samples taken by the inspectors under the local authorities in England and Wales under the sale of food and drugs act was 93,088. This included 43,794 samples of milk and 17,845 samples of butter. During this same period 6,842 total samples were taken in Scotland, 3,455 of these being milk.

An excellent plan is followed in the collection of informal test samples by the inspectors under the local authorities, and during 1907 in Scotland 1,733 test samples were taken, including 702 samples of milk and 384 samples of butter. Due to the nature of the collection of these samples, it is impossible for the local authorities to bring prosecution when samples are found to be adulterated, but it gives them a very good idea of the nature of the commodities being sold to the public and indicates where the inspectors may well collect official samples. In many instances it is found that in the collection of official samples by an authorized inspector great care and circumspection have to be exercised in order to obtain those similar to the ones which are as a rule offered to the persons regularly making purchases from the merchants and who are known to them. In the United Kingdom informal sampling is mainly done by local authorities as part of their ordinary administration. The collection of informal test samples is carried on by the Department of Agriculture in this country, and much valuable information is thus obtained. The difficulty experienced in Great Britain in the collection of official samples similar to the test samples is not experienced, fortunately, by the inspectors operating under the food and drugs act in the United States. In 1909 about 10 per cent of the official samples of all kinds collected in Scotland with the formalities required by the sale of food and drugs act were found adulterated, while the informal samples showed an adulteration equivalent to about 17 per cent. Official milk samples showed about 14 per cent adulteration and informal samples nearly 28 per cent adulteration. Of the official butter samples about 9 per cent were adulterated and of the test samples about 11 per cent.

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[1] Loc cit., p. 5.

In Great Britain each county, borough, or town council appoints one or more of its officers as inspectors to take samples and act under the sale of food and drugs acts. The direction of their work is usually a duty of the medical officer of health of the district. There are about 1,000 such inspectors, and they operate largely under the sale of food and drugs act, but have little to do with the butter and margarine act.

It is somewhat surprising to find that in a country where such careful control of dairy products is exercised there is not a great deal of activity in the sale of milk obtained from herds of tuberculin-tested cows. There appears to be practically no demand for certified milk, for while some attempts have been made along this line, they have failed of popular support.

From an administrative point of view one interesting phase of this work is seen in the circulars which are sent to the local authorities in Great Britain for the purpose of aiding them in the administration of the sale of food and drugs acts, 1875–1907. The Local Government Board issues a circular annually on administrative arrangements and also occasionally sends out special circulars relating to the adulteration of particular articles or to the reports of the board's inspectors of foods. Reference may be made by way of illustration to the circular issued under date of October 10, 1909, by the Board of Agriculture and Fisheries. This deals with the results of examination of the samples of products used for the thickening of cream. Two of the samples contained solutions of lime in cane-sugar sirup, that is, calcium sucrate, which is well recognized for its ability to thicken cream when used in small amounts. The determination of the sucrose and of the lime in the ash will reveal this adulteration. To illustrate the type of memoranda sent out by the Local Government Board to the local authorities, reference may be made to one issued on May 25, 1910, under the caption "Sampling under sale of food and drugs acts." This memorandum deals with the sale of lard substitutes as lard, the presence of water in lard substitutes, and the presence of paraffin in lard, lard substitutes, and margarine. Information of this kind from headquarters can not but be of inestimable value to the local authorities, as it keeps them in touch with the subjects under investigation and suggests what adulterants should be looked for.

DISCUSSION OF THE PRINCIPAL FOOD LAWS.

THE SALE OF FOOD AND DRUGS ACTS, 1875-1899.

The sale of food and drug acts, 1875-1899, and the butter and margarine act, 1907,[1] are the laws which are most interesting from the viewpoint of those who have to deal with the adulteration of food, and most of the prosecutions are based on these. The sale of food and drugs act, 1875, is the basic law. The amendment to it in 1899 makes a large number of changes. The most important of these changes are briefly as follows:[2]

> The changes in the law occasioned by the act of 1899, which comes into operation on the 1st of January next, are very considerable; the most important being (sec. 1) the precautions against importation of agricultural and other produce insufficiently marked; (sec. 2) power for the Local Government Board and Board of Agriculture to sample articles of food; (sec. 3) the imposition on local authorities of a legal duty to appoint analysts and exercise their powers under the food and drugs acts and power for the Local Government Board or Board of Agriculture to act in their default; (sec. 4) power for Board of Agriculture to make regulations and standards as to analysis of milk, cream, butter, or cheese; (sec. 7) provisions as to keeping of register by manufacturers and dealers in margarine and margarine cheese, and power for officers of the Board of Agriculture to inspect and take samples from manufactories of margarine and margarine cheese; (sec. 8) restriction on the amount of butter fat in margarine; (sec. 17) the increase of penalties and power in the court to imprison in certain cases; (sec. 26) the enlarged definition of food.

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[1] See pp. 11 and 22 for administrative details.

[2] The Adulteration of Food, by D. C. Bartley, 3d ed., 1907.

IMPORTANT SECTIONS OF THE ACT.

The great bulk of prosecutions in Great Britain under the sale of food and drugs acts, 1875–1899, occur under sections 3 to 9, inclusive. These sections are found in the 1875 act and the ones not modified in the act of 1899 are as follows:

3. *Mixing injurious ingredients with food.* No person shall mix, colour, stain, or powder, or order or permit any other person to mix, colour, stain, or powder any article of food with any ingredient or material so as to render the article injurious to health, with intent that the same may be sold in that state, and no person shall sell any such article so mixed, coloured, stained, or powdered, under a penalty in each case not exceeding fifty pounds for the first offence; every offence, after a conviction for a first offence, shall be a misdemeanor, for which the person on conviction, shall be imprisoned for a period not exceeding six months with hard labor.

4. *Mixing drugs with injurious ingredients.* No person shall, except for the purpose of compounding as hereinafter described, mix, colour, stain, or powder, or order or permit any other person to mix, colour, stain, or powder, any drug with any ingredient or material so as to affect injuriously the quality or potency of such a drug, with intent that the same may be sold in that state, and no person shall sell any such drug so mixed, coloured, stained, or powdered, under the same penalty in each case respectively as in the preceding section for a first and subsequent offence.

5. *Proof of absence of knowledge.* Provided that no person shall be liable to be convicted under either of the two last foregoing sections of this Act in respect of the sale of any article of food, or of any drug, if he shows to the satisfaction of the justice or court before whom he is charged that he did not know of the article of food or drug sold by him being so mixed, coloured, stained, or powdered as in either of these sections mentioned, and that he could not with reasonable diligence have obtained that knowledge.

6. *Sale of articles of food and of drugs not of the proper nature, substance, and quality.* No person shall sell to the prejudice of the purchaser any article of food or any drug which is not of the nature, substance, and quality, of the article demanded, by such purchaser, under a penalty, not exceeding twenty pounds; Provided that an offence shall not be deemed to be committed under this section in the following cases; that is to say

(1) Where any matter of ingredient not injurious to health has been added to the food or drug because the same is required for the production or preparation thereof as an article of commerce, in a state fit for carriage or consumption, and not fraudulently to increase the bulk, weight, or measure of the food or drug, or conceal the inferior quality thereof;

(2) Where the drug or food is a proprietary medicine or is the subject of a patent in force, and is supplied in the state required by the specification of the patent;

(3) Where the food or drug is compounded as in this Act mentioned;

(4) Where the food or drug is unavoidably mixed with some extraneous matter in the process of collection or preparation.

7. *Compound articles of food and compounded drugs.* No person shall sell any compound article of food or compounded drug which is not composed of ingredients in accordance with the demand of the purchaser, under a penalty not exceeding twenty pounds.

8. *Protection from offences by giving label.* Provided that no person shall be guilty of any such offence as aforesaid in respect of the sale of an article of food or a drug mixed with any matter or ingredient not injurious to health, and not intended fraudulently to increase its bulk, weight, or measure, or conceal its inferior quality, if at the time of delivering such article or drug he shall supply to the person receiving the same

a notice by a label distinctly and legibly written or printed on or with the article or drug, to the effect that the same is mixed.

9. *Abstraction of part of an article of food before sale.* No person shall, with the intent that the same may be sold in its altered state without notice, abstract from an article of food any part of it so as to affect injuriously its quality, substance, or nature, and no person shall sell any article so altered without making disclosure of the alteration, under a penalty in each case not exceeding twenty pounds.

PRINCIPAL OFFENSES.

These offenses are well condensed by Bell, Scrivener, and Lloyd in their book, The Sale of Food and Drugs Acts, 1875 to 1899, fourth edition, on pages 32 et seq., of the introduction, which read as follows:

PRINCIPAL OFFENCES.

(1)—(a) The mixing of injurious ingredients with any article of food or drug sold or intended to be sold (sections 3 and 4).

(b) The selling of any article so mixed (sections 3 and 4).

(2)—(i) The selling of any article of food or drug which

(a) is inferior in nature, substance, and quality to the article demanded by the purchaser (section 6).

(b) being a compounded article of food or drug, is not compounded in accordance with the demands of the purchaser (section 7).

(ii)—(a) The abstraction from any article of food sold or intended to be sold or any part of it, so as to affect injuriously its quality, nature or substance, without making disclosure of the alteration (section 9).

(b) The selling of any article so altered (section 9).

The main differences between the two classes of offences are, that in the case of (1) it must be shown that the article is injurious to health (or, if it be a drug, that its quality or potency has been injuriously affected), whereas in the case of (2) the fact that the addition, abstraction, or whatever it may have been was harmless, is immaterial; that in the case of (1) guilty knowledge is an essential element of the offence, whereas in (2) it is not; and that the penalty under (1) is, of course, much heavier.

That offences of the first class are comparatively rare nowadays, is shown by the following extract from the report of the Select Committee of 1894–1896:

"There is reason to think that the adulteration of food with substances injurious to health has diminished greatly during recent years. Proceedings have rarely been taken under Section 3 of the Sale of Food and Drugs Act, 1875, in respect of this class of offences. No doubt this may partly be accounted for by the fact that it would in no case be an easy matter to obtain convictions under the section referred to, because it is necessary to prove that the adulteration is dangerous to health, and that the person charged with the adulteration has guilty knowledge of the same. Moreover, it is to be borne in mind that the punishments which may be inflicted for offences of this character are more severe than those imposed by the provisions of the Act which relate to adulteration with harmless substances. But whatever may be the cause of the diminution of this description of offences, it is satisfactory to note the same."

For the second class of offences, section 6 is the all-important section, and under it the bulk of the prosecutions under the Sale of Food and Drugs Acts are instituted. Although its provisions have been made the subject matter of a number of reported cases, the tendency of the decisions throughout has been to widen, rather than to curtail, its scope. It is now well recognized that the primary object of these acts is not so much the punishment of those who deserve to be punished, as the protection of the public at large, and, therefore, the seller of an adulterated article is liable to a conviction under this section, even though he may be innocent of any attempt to cheat his customers, and though the actual offender (who can, of course, also be punished) may be a servant, or even a person over whom he has no control.

Section 7, which refers to compounded articles, is little used, inasmuch as an offence against its provisions is equally punishable under section 6.

Section 9 deals with abstraction alone, and was specially designed to meet the case of the removal of cream from milk. Its provisions are more stringent than those of section 6, and certain defences which are available under section 6, as will be seen presently, are not available under section 9.

COMPARISONS WITH THE FOOD LAW OF THE UNITED STATES.

Section 3 of the 1875 act resembles somewhat in its wording certain phraseology found in the food law of the United States, namely, section 7, paragraph 4, in the case of foods, where it is held that a food is adulterated "if it be mixed, colored, powdered, coated, or stained in a manner whereby damage or inferiority is concealed." The 1875 act, however, makes the offense depend upon the mixing, coloring, staining, and powdering with an ingredient so as to render the article injurious to health. Another very important difference lies in the phrase "with *intent* that the same may be sold in that state." Under this section a provision is made for a prison sentence not to exceed six months on a second conviction. The penalties provided for under the sale of food and drugs acts are such that the first offense bears a maximum penalty of £20, the second offense £50, and the third and subsequent offenses £100 each (sec. 17, act of 1899). Provision is also made in section 17 of the act of 1899 for imprisonment, where the "offense," in the opinion of the court, "was committed by the personal act, default, or culpable negligence of the person accused." The limit of imprisonment is three months.

Under our Federal law, where conviction is obtained in the courts for shipping misbranded or adulterated foods or drugs in interstate commerce, the limit of the penalty is a $200 fine for the first offense, and for each subsequent offense a fine not to exceed $300 or imprisonment not to exceed one year, or both, in the discretion of the court.

The penalty for the manufacturer of proscribed foods and drugs within the Territories and the District of Columbia is more severe than for their sale in interstate commerce, being for each offense a fine not to exceed $500 or one year's imprisonment, or both, within the discretion of the court.

In prosecutions under section 3 of the English law guilty knowledge must be proven. This is required by section 5. The courts of Great Britain have not been invoked to act frequently under section 3. The question of intent has decreased its usefulness, and again, trials based on it are found to be very expensive because of the expert evidence necessary to prove the effect on health of the articles of food under discussion. It is in connection with this section that the "Report of the departmental committee, appointed to inquire into the use of preservatives and coloring matters in the preservation and coloring of food" is of value. The report of this committee was published in 1901, and their recommendations are given on page 30.

In 1896 the justices at South London sessions had before them the question of sulphate of copper used in greening peas. They held that it was in violation of section 3 to sell a 1-pound bottle of peas with the knowledge that it contained an amount of copper equivalent to 3 grains of this chemical. This is equivalent to 0.8 grain of metallic copper per pound. (Sumners *v.* Grist, 60 J. P., 346.) There have in fact been other successful prosecutions in the courts of Great Britain because of the large amounts of copper found in peas and spinach. Among recent cases, reference may be made to spinach containing 10 grains of copper sulphate per pound (British Food Journal, 1910, p. 19), and peas containing 3.5 grains of copper sulphate per pound (British Food Journal, 1910, p. 99).

Under section 3 of the act of 1875 it is not sufficient to prove that the substance mixed with the food is injurious to health, but it is necessary to show that the food so prepared is injurious. A person can not be prosecuted for the sale of an injurious article not in itself a food, although the vendor had knowledge that the injurious substance was to be mixed with foodstuffs or used in their preparation.

In the law of the United States there are two requirements: (1) The added ingredient must be shown to be a poison or a deleterious substance; (2) the article containing the added poison or deleterious substance must be shown to be one that may be injurious to health.

Section 4 of the act of 1875 deals with drugs, and its phraseology is somewhat similar to that of section 3, which deals with foods. This difference is to be noted: Section 4 says nothing about the drug being injurious to health, but merely that the mixing, etc., shall "affect injuriously the quality or potency" of the drug, and section 5 relieves the vendor from responsibility if he can show the absence of knowledge of the fact that the potency or quality of the drug had been injured and "that he could not with reasonable diligence have obtained that knowledge."

The most important section of all is section 6, for under it most of the prosecutions are now brought. Under this section it is in violation of the sale of food and drugs act to "sell to the *prejudice* of the purchaser any article of food or any drug which is not of the *nature, substance*, and *quality* of the article demanded." One important feature, however, of the whole section which differentiates it from sections 3 and 4 is that it is not incumbent to prove guilty knowledge; that is, that the *vendor knew* that the purchaser was being prejudiced by purchasing a product not of the nature, substance, and quality demanded. This makes the provisions of these acts more readily enforced, and prosecutions may be brought under section 6 which might have been unsuccessful if brought under sections 3 and 4. In the administration of section 6 one phase of judicial construction is particularly interesting because it shows the difference in the method of handling cases in Great Britain and in the United States. In Great Britain "a purchaser can not be prejudiced when notice is given to him at the time of the sale that an article sold is not of the nature, substance, and quality of the article he demands." In the United States it is required that the label give proper notice; that is, if a product is labeled, it must carry truthful statements on it. There is, in general, no legal requirement for placing a label on a food, and in the case of drugs, only limited requirements. It is possible, however, for a food to be both adulterated and misbranded although bearing no label. The Secretary of the Treasury, the Secretary of Agriculture, and the Secretary of Commerce and Labor, in Regulation 22 of the Rules and Regulations for the Enforcement of the Food and Drugs Act (Circular No. 21, revised), state that—

> It is prohibited to sell or offer for sale a food or drug product bearing no label upon the package or no descriptive matter whatever connected with it, either by design, device, or otherwise, if said product be an imitation of or offered for sale under the name of another article.

There are, under section 6 of the sale of food and drugs act, four provisos which give exemption from its terms. Subsection 2 excludes proprietary medicines from the consideration of the law. Subsection 3 of section 6 is inoperative because the law is silent as to how drugs are to be compounded.

No standards are written into many of our State laws nor into the Federal law, and the same is practically true of the sale of food and drugs act[1] and it is necessary in consequence to determine in each specific case whether the article is in violation of section 6. The British Pharmacopœia is generally made use of in connection with the enforcement of the law, and though it is not conclusive, very strong evidence is necessary to displace it.

Subsection 4 of section 6 leaves it to be determined in each instance by evidence whether or not a food or drug has been unavoidably mixed with extraneous matter in the process of collection or preparation. This must be necessarily so in the absence of standards, and there is a complete parallel in the food and drugs act of the United States. While Circular 19 of the Office of the Secretary of Agriculture may serve as a guide in determining whether, for example, cayenne pepper has an undue amount of sand or dirt ground with it, yet it must be determined by the courts whether or not the amount found constitutes an adulteration.

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[1] There are a few exceptions in the sale of food and drugs acts, as water in spirits (1879 act, sec. 6); water in butter and margarine (butter and margarine act, 1907), etc.

This identical type of question has in fact come before the British courts and they determined that "owing to the method in which caper tea is produced, the presence of 3.5 per cent of mineral matter in the tea did not constitute an adulteration, and that the seller was protected by this subsection."

Section 7 is less broad in its language than section 6, and any article in violation of the former may be prosecuted under the latter. Section 7 seems to apply to those compounded goods, "the composition of which is distinctly recognized or indicated."

Section 8 is a labeling provision, and in effect provides that an article if distinctly and legibly labeled is not in violation of this section, unless the food or drug is mixed in a manner to increase its bulk, weight, or measure, fraudulently. With this latter proviso in view, the British courts have held that a mixture of 60 per cent of chicory and 40 per cent of coffee was, in spite of a label stating that the product was such a mixture, in violation of section 8, because the chicory has been fraudulently added to increase its bulk, weight, or measure. In this case, however, it appears that the inspector asked for coffee and received the product labeled as a mixture of chicory and coffee, which it was in fact. In another case, an inspector asked for "French coffee" and received a product so labeled, the label indicating further that the product was a mixture of chicory and coffee. The courts of Great Britain held that the label gave proper notice to the purchaser and that he was not prejudiced. In another case, where the purchaser asked for "coffee" he was informed that there was none in stock and was sold instead a product labeled "coffee and chicory," his attention having been called to the label prior to the purchase. The courts refused to convict in this case.

Section 9 has its counterpart in section 7 (3) in the case of foods in the food law of the United States, which considers a food adulterated "if any valuable constituent of the article has been wholly or in part abstracted."

Section 9 appears to have been included in the sale of food and drugs acts to cover especially those cases where milk has been deprived of the whole or a part of the butter fat or cream normal to it.

There has been a number of interesting milk cases brought under this section. For example, a product was labeled "condensed skimmed milk." Analysis showed that 97 per cent of the fat had been removed by means of a separator. Inasmuch as proof was adduced that not over 63 per cent of milk fat could be removed by skimming, the product was not properly designated as "condensed *skimmed* milk" and the high court did not interfere with the judgment of the lower court in this case, which had been for conviction. In another case a product was labeled "condensed milk, Swiss dairy brand"; in small type the label bore the further legend, "this tin contains skimmed milk." There was a deficiency of 93 per cent butter fat, but the court held that the purchaser had been sufficiently informed as to the character of the product although his attention had not been specifically called to the label at the time of purchase. Another interesting case is that of a vendor of milk who, over a period of four or five hours, sold milk in small amounts from a can containing 8 gallons of whole milk. The milk was not thoroughly mixed each time a sale was made and as the cream rose the lower part of the can contained a milk deficient in fat. When but 2 quarts remained, an inspector purchased a sample, which on analysis was found to show a deficiency of 33 per cent of fat and the courts decided that section 8 had been violated.

METHOD OF SAMPLING.

The method of sampling is very similar to that pursued by the inspectors under the American law. It is required that the samples, so purchased for analytical purposes, shall be divided into three parts at the time of purchase, and after sealing or otherwise marking each part the consignor "shall, if required to do so, deliver one of the parts to the seller or his agent." The consignor must, however, be informed at the time of purchase that the samples are to be analyzed by the public analyst. One of the remaining parts goes to the public analyst, the other is retained for "future comparison." If this third sample is lost or becomes spoiled so as to be unfit for analysis, the case can not be tried in court, for the court will require that the third sample be produced. The vendor can appeal for the third sample to be analyzed, and such analysis is made in the inland revenue laboratory. The vendor may have his sample analyzed by anyone he chooses. This brings about a very peculiar situation, namely, that it is possible for the public analyst to have for examination not only the official sample, but also the vendor's sample, because most public analysts are not exclusively engaged in the public service, but are engaged in outside work as well.

The regulations in this country require that "A sample taken from bulk goods shall be divided into three parts, and each shall be labeled with the identifying marks. If a package be less than 4 pounds, or in volume less than 2 quarts, three packages shall be purchased when practicable * * *. When three samples are purchased, one sample shall be delivered to the Bureau of Chemistry or to such chemist or examiner as may be designated by the Secretary of Agriculture; the second and third samples shall be held under seal by the Secretary of Agriculture, who, upon request, shall deliver one of such samples to the party from whom purchased or to the party guaranteeing such merchandise. When it is impracticable to collect three samples or to divide the sample or samples, the order of delivery outlined above shall obtain, and in case there is a second sample the Secretary of Agriculture may, at his discretion, deliver such sample to the parties interested. All samples shall be sealed by the collector with a seal provided for the purpose."

In the United Kingdom the Local Government Board, as well as the Board of Agriculture, has power to collect samples under the sale of food and drugs act, and when they are collected "the officer procuring such sample shall divide the same into four parts and shall deal with three of such parts in the manner directed by section 14 of the sale of food and drugs act, 1875, as amended by this act and shall send the fourth part to the board * * *." This is taken from section 2 of the sale of food and drugs act, 1899. The amendment referred to (sec. 13 of the act of 1899) is discussed above and differs but little from the form in which it is found in the 1875 statute.

There exists, however, one marked difference in the mode of collecting small samples. In Great Britain the separation or subdivision must be made from a sample comprising the whole amount purchased. In one instance an inspector purchased six bottles of camphorated oil which were subdivided into three sets of two bottles each. The court held that this was not the correct procedure and that the samples were illegal. What should have been done was to mix the entire contents of the six bottles and then subdivide as required by the law.

It should be noted in passing that the sale of food and drugs act provides a penalty for refusing to sell an article to any officer.

The certificate of analysis and findings of the public analyst are considered as evidence in the courts, but the analyst is always subject to call for testimony at the wish of the defendant or prosecutor. While this is specifically provided for in the sale of food and drugs act, 1875 (sec. 21) and 1899 (sec. 22), when certificates are submitted by both parties interested in the prosecution and the results given are more or less contradictory, it is left to the magistrate to determine which is the more reliable. If the difference is a matter which can be determined by analysis of the third or reserve sample, the magistrate in such cases adjourns the case for a report on that sample by the principal chemist of the Government (inland revenue) laboratory.

GUARANTIES.

One of the important sections of the act of 1875 is section 25, as amended by section 20 of the act of 1899. These sections deal with the question of warranty. The act of 1875 (sec. 25) provides that:

> If the defendant in any prosecution under this act prove to the satisfaction of the justices or court that he had purchased the article in question as the same in nature, substance, and quality as that demanded of him by the prosecutor, and with a written warranty to that effect, that he had no reason to believe at the time when he sold it

that the article was otherwise, and that he sold it in the same state as when he purchased it, he shall be discharged from the prosecution, but shall be liable to pay the costs incurred by the prosecutor, unless he shall have given due notice to him that he will rely on the above defense.

The amendment (sec. 20 of the act of 1899) changes the scope of the above somewhat, but does not render negative the principle that a warrant under such certain conditions is in itself a good defense. This principle has also been included in the food law of the United States under section 9, wherein a guaranty may be used as a defense provided it comes from the person from whom the goods were purchased. In England the primary object was to protect the innocent retailer, as under the English sale of food and drugs acts the prosecutions are always first instituted against the dealer. The English warranty provisions, while protecting the retailer, are defective against the wholesaler or manufacturer, and there is at present a considerable demand from local authorities for legislation which will enable them more effectively to reach the responsible person. Section 20 (3) of the act of 1899 provides that—

> A warranty or invoice given by a person resident outside the United Kingdom shall not be available as a defence * * * unless the defendant proves that he had taken reasonable steps to ascertain and did in fact believe in the accuracy of the statement contained in the warranty or invoice.

Herein is a distinction to be drawn between the warranty section of the sale of food and drugs act, 1899, and that of the American law, section 9 of which provides that the guaranty must be signed by a party residing in the United States.

SALE OF MILK REGULATIONS, 1901.

By virtue of the authority given the Board of Agriculture in section 4 of the sale of food and drugs act (1899), it has issued what is termed the "Sale of milk regulations, 1901." When milk contains less than 3 per cent of milk fat or less than 8.5 per cent of solids not fat, it lies with the seller to prove that water has not been added. The same is true in the case of skimmed milk containing less than 9 per cent of total milk solids. Under the same authority (sec. 4) the Board of Agriculture has issued the sale of butter regulations, 1902. These provide that if butter contains more than 16 per cent of water, it rests with the seller to prove that water was not intentionally added. It is established that milk may contain under certain circumstances less than 3 per cent of milk fat and still be genuine; on the contrary, milk may have over 3 per cent of milk fat and not be a genuine milk. The sale of milk (Ireland) regulations, 1901, and the sale of butter (Ireland) regulations, 1902, are identical with those discussed above for Great Britain.

In 1907 a request was made of the Board of Agriculture and Fisheries for legislation making it compulsory for local authorities, in cases where milk was found below the standards set forth in the sale of milk regulations, 1901, to resort to the procedure known as the appeal to the cow. This procedure was to consist in the collection of fresh samples within 24 hours after the inspector had collected the suspected milk, due regard being given to the amount of milk sent out by the cow keeper on the day of the appeal as compared with the amount sent out on the day on which the sample was collected by the inspector. The Board of Agriculture and Fisheries does not appear to favor this appeal to the cow being made compulsory, for there are apparently serious administrative difficulties which would render such legislation unwise. It appears, however, that when this practice has been voluntarily instituted by the several local authorities, some useful results have been obtained.

BUTTER AND MARGARINE ACT, 1907.[1]

On the 1st of January, 1908, the butter and margarine act, 1907, went into effect. This act is a part and parcel of the sale of food and drugs acts, but deals exclusively with the control of margarine, butter, and "milk-blended" butter. By the term "margarine" is understood "any article of food, whether mixed with butter or not, which resembles butter and is not milk-blended butter." "Milk-blended butter" is any mixture produced by mixing or blending butter with milk or cream other than condensed milk or cream."

The butter and margarine act, 1907, provides for the power of entry at all reasonable times by Government officers upon "premises registered under the sale of food and drugs acts or this act, and to inspect any process of manufacture, blending, reworking, or treatment used therein, and to take samples for analysis of any butter, margarine, margarine cheese, milk-blended butter, or of any article capable of being used in the manufacture, treatment, or adulteration of any such article aforesaid." Margarine cheese is defined in section 25 of the sale of food and drugs act, 1899, as "any substance, whether compound or otherwise, which is prepared in imitation of cheese, and which contains fat not derived from milk."

If any substance is found on the premises of a butter factory intended for use in adulterating butter, the one occupying such premises is guilty of an offense under the act. A legal maximum limit of 16 per cent of water is placed on butter in cases where butter is prepared for sale or consignment in a butter factory, which butter has been made, blended, reworked, or treated in the factory. Milk-blended butter must not contain over 24 per cent of water. It is likewise an offense to import into the United Kingdom butter containing more than 16 per cent of water, margarine having more than 16 per cent of water and 10 per cent of butter fat, or milk-blended butter having in excess of 24 per cent of water. The importation of any of these products containing preservatives prohibited by any regulation made under this act, or any more of a preservative than may be permitted under such regulations, is forbidden. The preparation of regulations for the use of preservatives is placed in the hands of the Local Government Board, and such regulations apply as well to sales within the United Kingdom as to importations.

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[1] See p. 22 for administration.

That portion of the butter and margarine act, 1907, which applies to imports is merely an addition to the power given the commissioners of customs under the sale of food and drugs act, 1899, in section 1. In this connection it is of interest to note that this section 1 requires all "condensed separated or skimmed milk" offered for import, to bear in large and legible type the legend "machine-skimmed milk," or "skimmed milk," as the case may require.

This act defines clearly how margarine must be labeled, how invoiced, and how advertised. In brief, it must be called "margarine," though this name may be combined with some descriptive or fancy name, which, however, must have the approval of the Board of Agriculture and Fisheries. This board must likewise approve of the names under which milk-blended butter may be sold.[1] Another provision of this act is that which requires the wrappers carrying such a product to bear in an approved form a statement of its water content. This law likewise forbids the Board of Agriculture and Fisheries from approving any name to be used in connection with the sale of margarine or milk-blended butter, which "refers to or is suggestive of butter or anything connected with the dairy interest." The total number of butter factories registered under the butter and margarine act, 1907, on December 31, 1908, was 159; of margarine factories, 30; and of factories for mixing or blending butter and milk, 12.

In the United States there are specific laws covering oleomargarine (or margarine, as it is called in the United Kingdom) and adulterated and process or renovated butter. The oleomargarine law is the act of August 2, 1886 (24 Stat., 209), as amended by acts of October 1, 1890 (26 Stat., 621), and May 9, 1902—

> to make oleomargarine and other imitation dairy products subject to the laws of any State or Territory, or the District of Columbia, into which they are transported, and to change the tax on oleomargarine, and to impose a tax, provide for the inspection, and regulate the manufacture and sale of certain dairy products, and to amend an act entitled "An act defining butter, also imposing a tax upon and regulating the manufacture, sale, importation, and exportation of oleomargarine." Approved August 2, 1886.

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[1] In a similar manner the Secretary of Agriculture either approves or disapproves of labels to be used on "renovated" or "process" butter.

This act and its amendments are enforced by the Commissioner of Internal Revenue of the Treasury Department, who has the authority given him by law to "make all needful regulations for the carrying into effect of this act." His regulations must, however, receive the approval of the Secretary of the Treasury. (Sec. 20, act of Aug. 2, 1886.)

In section 5 of the act of May 9, 1902, the Secretary of Agriculture is—

> authorized and required to cause a rigid sanitary inspection to be made, at such times as he may deem proper or necessary, of all factories and storehouses where process or renovated butter is manufactured, packed, or prepared for market, and of the products thereof and materials going into the manufacture of the same. All process or renovated butter and the packages containing the same shall be marked with the words "renovated butter" or "process butter" and by such other marks, labels, or brands, and in such manner as may be prescribed by the Secretary of Agriculture, and no process or renovated butter shall be shipped or transported from its place of manufacture into any other State or Territory, or the District of Columbia, or to any foreign country, until it has been marked as provided in this section. The Secretary of Agriculture shall make all needful regulations for carrying this section into effect, and shall cause to be ascertained and reported from time to time the quantity and quality of process or renovated butter manufactured, and the character and the condition of the material from which it is made. And he shall also have power to ascertain whether or not materials used in the manufacture of said process or renovated butter are deleterious to health or unwholesome in the finished product, and in case such deleterious or unwholesome materials are found to be used in products intended for exportation or shipment into other States or in course of exportation or shipment he shall have power to confiscate the same.

Regulations No. 9, revised July, 1907, of the United States Internal Revenue, contains the "Revised regulations concerning oleomargarine, also adulterated butter and process or renovated butter." These regulations have not been greatly modified, but where such revision has taken place they are published as Treasury Decisions (as, for example, T. D. 1498).

As illustrating the fact that the problems with which those who administer the food laws of the United Kingdom have to deal are the same as those that are being dealt with in the United States, reference may be made to the difficulties which the Board of Agriculture and Fisheries has met with in approving of names for margarine. The annual report of the intelligence division of this board for the year 1907 (p. 15) says:

> The board also declined to approve of words or phrases importing praise, except praise of a kind which is not applicable to margarine, and of words which imply that the margarine is suitable for any particular purpose. The necessity for this precaution has been already fully demonstrated, as more than one firm has attempted to refer to the board's approval of names on wrappers and in advertisements in such a way as to convey to the purchaser the idea that the margarine itself had been approved by the board.

The same type of difficulty is encountered in the United States, where the guaranty legend is often said to represent the Government's guarantee of the quality of the article on which such a legend is placed. Unfortunately it is so looked on by many, and this view is undoubtedly fostered by the unscrupulous. Food Inspection Decision 99, amending Regulation 9 of the food and drugs act, was issued to minimize this difficulty, and requires that the name of the guarantor be inserted in the guaranty legend on the label.

A long step forward has been made by this butter and margarine act, 1907, by controlling the name by which margarine is described in an advertisement.

FERTILIZERS AND FEEDING STUFFS ACT, 1906.

It is through the fertilizers and feeding stuffs act, 1906, that control is had of feeding stuffs for animals other than man. The gist of this act, apart from the portion relating to fertilizers, is found in section 2, which states that—

> (2) Every person who sells for use as food for cattle or poultry any article which has been artificially prepared shall give to the purchaser an invoice stating the name of the article, and whether it has been prepared from one substance or seed or from more than one substance or seed, and in the case of any article artificially prepared otherwise than by being mixed, broken, ground, or chopped what are the respective percentages (if any) of oil and albuminoids contained in the article, and the invoice shall have effect as a warranty by the seller as to the facts so stated, except that as respects percentages the invoice shall have effect as a warranty only that the actual percentages do not differ from those stated in the invoice beyond the prescribed limits of error.

When an article is sold for feeding purposes it is supposed to be suitable for the use to which it is to be put, and to contain no other materials than those indicated by the name or description under which it is sold. Upon payment of a stipulated fee, any purchaser may receive a Government analysis of any feeding stuff if a sample is submitted within 10 days of the delivery or receipt of invoice.[1] An official sampler is also required to take the samples for analysis, at the request of the purchaser, but such samples may also be taken without such request. Three samples are taken when there is a possibility of civil or criminal proceedings being instituted, and, after sealing, the official sampler must deliver one of them to the seller. This plan of delivering one sample to the seller was followed in the administration of the United States food and drugs act, but it was found in some cases to be impracticable and useless, as prosecutions lie against vendors in States only when they sell in "original packages" as defined by the Supreme Court with respect to articles of interstate commerce. In the United States, when a request is made for an official sample, it is always given if practicable; the fertilizers and feeding stuffs act, 1906, renders such a procedure obligatory. As in proceedings under the sale of food and drugs act, the certificate of the analyst is sufficient evidence of the facts therein stated, unless the defendant makes a specific request for the appearance of the analyst.

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[1] In some of our States provision is made for the State analyst to make such analysis upon payment of the required fee.

It is forbidden to sell any feeding stuff without an invoice unless there is a reasonable excuse therefor. Such invoices shall not be "false to any material particular to the prejudice of the purchaser." Neither shall the feeding stuff contain an article dangerous to cattle or poultry nor have added to it "any ingredient worthless for feeding purposes and not disclosed at the time of the sale."

An excellent provision limits the time within which a prosecution may be brought "for an offense of causing or permitting an invoice or description to be false in any material particular." No prosecution can be instituted "after an expiration of three months from the date when the invoice was received by the purchaser." The rapidity with which cases must be carried to the courts under the sale of food and drugs acts is evidenced by section 19 of the 1899 act, wherein it is provided as follows:

> *Time for proceeding and regulation as to summons.*—(1) When any article of food or drug has been purchased from any person for test purposes, any prosecution under the sale of food and drugs acts in respect of the sale thereof, notwithstanding anything contained in section 20 of the sale of food and drugs act, 1875, shall not be instituted after the expiration of 28 days from the time of the purchase.

A number of regulations have been issued under the fertilizers and feeding stuffs act, 1906. They contain much of interest and value, dealing as they do with the practical side of the administration of this act. For example, the fertilizers and feeding stuffs (methods of analysis) regulations, 1908, deal with the details of analysis of feeding stuffs and discuss the preparation of the sample, determination of moisture, determination of oil, and determination of albuminoids. Such methods are official and especially desirable because they give a standard which is used by each of the analysts, thus producing comparable results. These methods have their counterpart in the methods of the Association of Official Agricultural Chemists.[1]

The fertilizers and feeding stuffs (sampling, etc.), regulations, 1906, dated December 27, 1906, deal more in details relative to the methods of sampling. The difficulty of procuring satisfactory samples of feeding stuffs is well recognized, and standard methods are particularly useful because of the greater ease of obtaining uniform analytical figures.

Another regulation which works for uniformity of interpretation is known as the fertilizers and feeding stuffs (limits of error) regulations, 1910, dated January 25, 1910. These limits of error apply to the statement of the percentages of oil and albuminoids as found in the invoice accompanying the sale of feeding stuffs and they vary with the class of feeding stuffs concerned. In cotton-seed cake or meal the analysis may vary one-tenth of the percentage of oil or albuminoids stated in the invoice, in such products as linseed cake or meal the variation may be as great as one-eighth, and in other types of feed it may even be one-fifth. The limits of error only affect civil proceedings for damages consequent on breach of warranty. The warranty in the invoice is that the actual percentages do not differ from those stated in the invoice beyond the prescribed limits of error. If the limit of error is 1 per cent and the deficiency is 3 per cent, civil proceedings can be taken for the recovery of the value of 2 per cent. Proceedings could be instituted under section 6 even though the deficiency was not beyond the limits of error, but such a case is not likely to occur in practice. These limits of error are generous and exceed distinctly the limits used in the administration of the food and drugs act by the Department of Agriculture in its control of similar products.[1] It might be pointed out that under the head of albuminoids, ammoniacal and nitric nitrogen should not be, and in fact are not, included.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Revised.

During the year 1909 there were 1,995 samples of feeding stuffs collected in England, Scotland, and Wales.

THE PUBLIC HEALTH ACT, 1907.

What is destined to be one of the most valuable of the food-control laws of Great Britain is the public health (regulations as to food) act, 1907. This act is principally of value in that power is given to make regulations authorizing measures to be taken for the prevention of danger arising to public health from the importation, preparation, storage, and distribution of articles of food or drink (other than drugs and water) intended for sale for human consumption. This act also contains the very essential requirement that articles commonly used for the food or drink of man shall be deemed to be intended for sale for human consumption unless the contrary is proved. This would apply to all those cases which are so often difficult to judge, namely, where the product has a dual use, first, for food, and second, for technical purposes. There are undoubtedly cases where a product fit only for the second class has been used for the first, and it would appear the part of wisdom to rest the burden of proof, as to the use to which a product is to be put, on the dealer. The administration of this act is in the hands of the Local Government Board.

The first regulation issued under this act was the public health (first series, unsound food) regulations, 1908. These deal with the control and handling of food from foreign countries which is unsound and unwholesome and unfit for consumption. Inspection takes place at the port of entry. Similar work had formerly been locally carried on at the port of London under the port sanitary authority and also at the port of Manchester, but such control had not been general until the issue of these regulations. When such unsound food is discovered by the medical officer of health of the port sanitary authorities, and the importer does not surrender it for destruction or dispose of it in a manner approved by the medical officer of health, an application for an act of condemnation is made of a justice, who may order the destruction of such food or provide for its disposal, except as an article for human consumption.

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[1] Five per cent, in case the protein, fat, or crude fiber content exceeds 20 per cent, and 8 per cent when the protein content is less. The percentage of these ingredients found by analysis must not, however, run uniformly below the stated percentages, by the amount set by these limits.

There have also been established public health (foreign meat) regulations, 1908. These regulations have for their object the control of the character of meat of various kinds coming from foreign countries and in general "to provide means by which foreign meat generally which is diseased, unsound, unwholesome, or unfit for human consumption may be detected and dealt with by the public health authorities at the place of importation." Such regulations are especially necessary in Great Britain because of the reliance that must be placed on importations to furnish an adequate food supply.

Official recognition has been given to certain countries respecting their inspection of meat obtained from cattle or pigs. When such meat products bear an official tag and come from Denmark, the Netherlands, the Dominion of Canada, or the Dominion of New Zealand they are recognized in general as coming from animals free from disease, as having been prepared and packed properly, and as not offering any danger to public health. The fact that such products are properly labeled, while giving facilities in the matter of inspection at the ports of entry, does not, however, grant any immunity from such inspection. These regulations virtually forbid the importation into the United Kingdom of a few specified articles of food, among which are included meat in the form of scraps, not identifiable with definite parts of the carcass; tripe, tongues, and kidneys which have been treated with preservatives, including boric acid; and fresh or frozen pork, when in portions less than the entire carcass, if not inclosed in a receptacle bearing an accepted certificate as to inspection in the country of origin.

THE MERCHANDISE MARKS ACTS, 1887-1894.

The merchandise marks act of 1887 makes it an offense, among other things, for any person to apply any false trade description to goods, unless "he proves that he acted without intent to defraud." (Sec. 1.) Not only does this act apply to the manufacturers, but likewise to "every person who sells, or exposes for, or has in his possession for, sale, or any purpose of trade of manufacture" any such goods. The expression "trade description" as defined by the act means "any description, statement, or other indication, direct or indirect, (*a*) as to the number, quantity, measure, gauge, or weight of any goods, or (*b*) as to the place or country in which any goods were made or produced, or (*c*) as to the mode of manufacturing or producing any goods, or (*d*) as to the material of which any goods are composed, or * * *."

These brief quotations from the merchandise marks act, 1887, show at once their applicability to the sale of food and drugs acts, and these principles have been used in the prosecution of manufacturers and dealers guilty of misbranding of their wares. The term "false trade descriptions" is also described in the act to apply to the use of any false or fictitious name. As illustrative of the skillful method used in handling certain types of trade descriptions, namely, those where foreign names are used, the whole of section 18 is here given:

> 18. Where, at the passing of this act, a trade description is lawfully and generally applied to goods of a particular class, or manufactured by a particular method, to indicate the particular class or method of manufacture of such goods, the provisions of this act with respect to false trade descriptions shall not apply to such trade description when so applied: *Provided*, That where such trade description includes the name of a place or country, and is calculated to mislead as to the place or country where the goods to which it is applied were actually made or produced, and the goods are not actually made or produced in that place or country, this section shall not apply unless there is added to the trade description, immediately before or after the name of that place or country, in an equally conspicuous manner, with that name, the name of the place or country in which the goods were actually made or produced, with a statement that they were made or produced there.

An application of the principle here laid down appears on page 20 of this bulletin, wherein is given the rule for labeling port and sherry wines produced in other countries than Portugal or Spain.

The merchandise marks act, 1887, was amended in 1891 and again in 1894, but the amendments are more commonly in connection with the administrative features of the 1887 act.

That the merchandise marks act may be of service in protecting the public against food which has been misbranded is shown by the work of the Board of Agriculture and Fisheries, which has its enforcement in hand as regards agricultural produce. Reference, for example, may be made to a prosecution brought at the instance of the Department of Agriculture and Technical Instruction for Ireland. A firm of provision merchants were successfully prosecuted for selling as "Irish" bacon a product of American origin. This department also has an inspector established in Great Britain to protect the interests of the Irish agricultural products. This step was taken because it appeared that the Irish trade had suffered materially because of frauds which had been practiced. This action of the

Irish authorities has already produced good results and undoubtedly still more marked results will be obtained in the future.

Under the merchandise marks acts attention has also been paid to such food products as butter, cheese, cider, and condensed milk, which were misbranded. During the year 1909 successful prosecutions were instituted for the sale of foreign poultry as English, foreign eggs as Irish, beverages falsely labeled as cider and perry, and foreign vegetables as English.

ACKNOWLEDGMENTS.

An attempt has been made in this bulletin merely to outline some of the important phases of the sale of food and drugs acts and those allied laws which make for the control of foods and drugs and for the protection of the consumer. Full acknowledgment is hereby given for the information obtained from various official publications, as well as from the various treatises which have been published on this subject. Acknowledgment is also made to those administrative officers through whose courtesy I was enabled to obtain much valuable information during an investigation of this subject made during the summer of 1908. These officials, both in England and Ireland, whom I have had the opportunity of consulting are accomplishing a splendid work. The problems confronting them in matters concerning the protection of the public health and the prevention of fraud are essentially those which are being dealt with in the United States. In this country we try to accomplish under our one law what they accomplish under many, but we have had the advantage in having the more recent legislation and have been able to take advantage of their experience. Then, too, conditions are not absolutely parallel, because the authority of the States is here clearly and distinctly outlined, while in Great Britain there is no such broad line of demarcation.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 144.

H. W. WILEY, Chief of Bureau.

WOOD TURPENTINE:

ITS PRODUCTION, REFINING, PROPERTIES, AND USES.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., July 31, 1911.

Sir: I beg to submit for your inspection and approval the results obtained in an investigation made in this bureau by F. P. Veitch and M. G. Donk on the distillation of resinous woods, especially the product wood turpentine. The investigation has been in progress for several years, and although certain phases on which work is being continued are not complete, it is deemed advisable to publish at this time the results so far obtained. Many inquiries are received for information on the subject of resinous-wood distillation, and it is believed that the data herein given will be helpful, and also will further promote the profitable utilization of large quantities of waste wood.

I recommend that this report be published as Bulletin No. 144 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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WOOD TURPENTINE:
ITS PRODUCTION, REFINING, PROPERTIES, AND USES.

INTRODUCTION.

Wood turpentine, "stump turpentine," or "wood spirits of turpentine," as the product is variously known, is turpentine oil made from cut pine, fir, or spruce, by distilling the wood in closed retorts. When properly refined it closely resembles gum spirits of turpentine, obtained by distilling the gum which oozes from the cut surface of the living tree. The turpentine made by distilling the wood with steam below a temperature of 150° C. more closely resembles gum spirits than that obtained by destructively distilling the wood. The latter is always contaminated with other oils derived from the breaking down of the wood and the resins which it contains. In some cases where the context shows plainly that wood turpentine is meant the term "turpentine" is used to avoid repetition; it should be noted, however, that commercially wood turpentine should always be designated as such and not as "turpentine."

Wood turpentine is used chiefly as a varnish and paint thinner as a substitute for, or in the place of, gum turpentine. Its production and use has increased slowly, partly because of its objectionable odor, its physiological effect, its lack of uniformity, and unsatisfactory working qualities due thereto, and partly because of the lack of knowledge regarding its ultimate effect on the color and durability of varnishes and paints.

The recovery of turpentine by distillation from the wood was attempted years ago, and the first patents relating to this subject were granted as early as 1841. The process was not developed, however, and it is only within recent years that any material success has attended its use. There are two primary causes for the numerous efforts which have been made during the past 10 or 15 years to recover turpentine from cut pine wood: First, the difficulty of supplying the increasing demand for turpentine, owing to the rapid decrease of the supply of live pine timber suitable for even the most advanced system of turpentine orcharding; second, the demand for a profitable method of utilizing the mill and forest wastes of the pine regions, not only that such wastes may be made valuable, but that the damage arising from their nonuse may be prevented. Thus it happens that business men and inventors have been actively devising and exploiting apparatus and processes for the recovery of the turpentine from this waste wood, and to distinguish it from ordinary gum turpentine the product is quite properly spoken of as wood turpentine or stump turpentine.

Judging from statistics, the production of gum spirits of turpentine in this country is now on the decline, as the census reports show that in 1909, 28,941,000 gallons; in 1908, 36,589,000 gallons; in 1907, 34,180,800 gallons; in 1905, 30,170,499 gallons; and in 1900, 38,488,170 gallons were produced. On the other hand, the value of the turpentine was $12,654,000 in 1909; $14,112,400 in 1908, $18,283,300 in 1907; $15,170,999 in 1905, against $14,960,235 in 1900. The pine timber available for turpentining is rapidly being exhausted, as is quite generally recognized among turpentine operators, and under present conditions no material permanent increase can be expected in the output of gum turpentine. This fact, as has been stated, is one of the chief reasons for the interest which has developed with regard to wood turpentine. If we accept the conclusion that the demand for turpentine can not be fully supplied from the present sources, it is reasonable to assume that if wood turpentine may be used for the same purposes, or for some of the purposes, for which gum spirits is used, it should find a ready market. The turpentine-using industries have been slow to accept wood turpentine, and although there is a market, it is still unfavorable, largely because of the nonuniformity of the product.

The owner of pine lands and the lumberman are directly interested in wood turpentine from another point of view, that of the profitable utilization of waste wood. In general, such methods of using wood are less profitable than to dispose of it through the usual channels, but the method utilizes material of little value for other purposes, which, when unused, is a source of danger from forest fires. Furthermore, the profitable utilization of waste timber and stumps makes it possible to clear cut-over lands at a minimum expense and prepare them for cultivation.

While it is well known that there are immense wastes of wood incident to the lumber industry, there are so few exact data that no accurate calculation can safely be made either as to the waste at the mill, including slabs and sawdust, or in the forest, including tops, laps, culls, dead-and-down timber, and stumps. Some figures on the subject, however, may not be without interest: The Census Bureau estimates that in 1908 approximately 11,000,000,000 board feet of southern yellow pine were cut; of Douglas fir, 4,000,000,000 board feet; and of western pine 1,225,000,000 board feet, a total of approximately 16,000,000,000 board feet. If the waste at the mill and in the forest be placed at 50 per cent of this, a very conservative estimate, there are 8,000,000,000 board feet, or approximately 8,000,000 cords of wood (on a basis of 40 per cent unoccupied space in a cord), available for the recovery of turpentine and rosin and the manufacture of paper, acetate of lime, wood alcohol, oxalic acid, etc. From this class of material from 1 to 10 gallons of crude turpentine per cord are recovered under the present practice, the average recovery being 3 gallons per cord.

Pine wood also yields from 3 to 15 or 20 per cent of rosin, while exceptionally rich lightwood may yield from 30 to 35 per cent. The average rosin yield of good lightwood is probably between 12 and 18 per cent. Assuming low yields of turpentine and rosin, a calculation shows that it is possible to recover from the wastes of the yellow-pine lumber industry (including dead-and-down timber) as much or more turpentine, rosin, and rosin oils as are now produced by the ordinary methods of turpentining from the living tree. The profitable utilization of mill wastes in this way would add materially to the wealth of the South and help to conserve its timber resources.

METHODS OF PRODUCTION.

Wood turpentine can be recovered from any of the coniferous woods. Those which have been found to contain sufficient resin to justify working them are the long-leaf yellow pine, of the South, the Norway pine of the Central North, and the Douglas fir of the Northwest. The methods by which wood turpentine is recovered may be appropriately divided into three classes, (*a*) destructive distillation methods, (*b*) steam distillation methods, (*c*) extraction with solvents.

DESTRUCTIVE DISTILLATION.

In the destructive distillation method wood exceptionally rich in turpentine, and rosin (generally termed "lightwood" or stumps) is loaded into retorts holding one or more cords each, and slowly heated at a low temperature until all the turpentine and other low-boiling oils are driven off and condensed, when the oils are directed to other receiving vessels, and the distillation of the wood continued until nothing remains in the retort but charcoal. In conducting this process it is important that the temperature of the retort should not rise above 200° C. until all turpentine in the wood has been driven off, otherwise the wood is decidedly charred and the turpentine is contaminated with other materials from which it can not be subsequently purified. Industrially, it has been found impracticable until very recently to prevent such contamination, and for this reason destructively distilled turpentine has certain characteristic properties which distinguish it from turpentine prepared in other ways. The time for distilling by the destructive process varies from 24 to 30 hours. Preliminary experiments conducted in the laboratory several years ago indicated that it is feasible to control the heating of the wood by using an oil jacketed retort, but no work on a commercial scale has been done with such apparatus as far as is known.

The following diagram shows the products of the destructive distillation of wood and the processes used:

- Resinous wood (distilled)
  - Charcoal.
  - Gases.
  - Tar
    - Used as such.
    - Distilled
      - Light oils.
      - Heavy oils.
      - Pitch.
      - Acid water.
  - Condensable distillate.
    - Light oils (fractionated)
      - Impure light rosin spirits.
      - Destructive wood turpentine.
      - Pine oils (includes heavier portion of rosin spirits).
    - Heavy oils (fractionated)
      - Pine oils (includes heavier portion of rosin spirits).
      - Rosin oil.
      - Pitch.
    - Aqueous distillate (neutralized with milk of lime and redistilled.)
      - Residue (dried and roasted)—acetate of lime.
      - Distillate (rectified)—wood alcohol.

STEAM DISTILLATION.

For distilling with steam the procedure and equipment are quite different from those used in destructive distillation. In the first place mill waste, including sawdust, can often be profitably utilized. Other wood must be chipped or cut into small pieces in order that the steam may penetrate it and remove the oils readily. The chipped wood, prepared by what is known as a "hog," is taken directly to the retorts by conveyers, or is placed in one of the many forms of inner containers which have been devised to facilitate the penetration of the chipped wood by the steam, and also the emptying of the retort at the conclusion of the distillation, both of which operations, even under the most favorable conditions, are quite difficult. Live steam is conducted into the retort until no more oils pass over, which requires in most plants from 3 to 24 hours. The distillate, consisting of a mixture of oils and water, is condensed, allowed to separate into two layers, and the crude oil is stored in large storage tanks, from which it is drawn to the refining still.

The live steam which is turned into the filled retorts may be superheated or saturated, and used at atmospheric pressure, under additional pressure, or at diminished pressure, as may be desired. All of these methods of handling the steam are used, but as a matter of fact, it can not be said that under the conditions at present usually obtaining in the industry, higher or better yields are secured by one procedure than by the other. As turpentine distills with steam at from 94 to 98° C., no advantage as to yield can logically be expected from the use of superheated or pressure steam, other than that which may arise from a quicker and more thorough penetration of the wood by the steam (which, as has been said, is difficult to secure) coupled with the smaller quantity of vapor to be condensed. Further, the use of steam under pressure is questionable, as the terpenes are readily polymerized at high temperatures. On the other hand, it is relatively certain that owing to the difficulty of securing contact, the usual steam process in fixed retorts does not remove all turpentine from the wood, and an apparatus or process which will insure contact of the steam with all of the wood will give higher yields of turpentine. In this connection, the use of a rotating retort immediately suggests itself, and several plants claim to obtain larger yields with these. Distillation under reduced pressure, if found practicable, undoubtedly offers several important advantages, among which are the distillation of turpentine at low temperature, thus reducing the tendency to polymerization, penetration of the wood by steam, and distillation of the turpentine from the interior of the pieces of wood.

Prolonged heating is exceedingly costly, and with proper equipment is needless, as it has been demonstrated that turpentine may be removed in from two to three hours, when proper penetration of the wood by the steam is secured. The steam process is particularly applicable for the utilization of mill wastes, such as slabs and sawdust, and has found its greatest development in connection with the sawmills of the South.

The following diagram shows the products of the steam distillation of wood and the utilization of the extracted chips:

- **Resinous wood** (hogged and steam distilled)
  - Distillate
    - Water.
    - Crude oils (fractionated)
      - Steam wood turpentine (60 to 80 per cent).
      - Pine oils (20 to 40 per cent).
  - Chips
    - Destructively distilled (see p. 10).
    - Wasted or burned.
    - Digested with caustic soda and drained
      - Paper pulp.
      - Crude light oils (removed during digestion and fractionated)
        - Steam wood turpentine.
        - Pine oils.
      - Black liquor (concentrated and destructively distilled)
        - Sodium carbonates (washed out of charcoal).
        - Products (see p. 10).

EXTRACTION WITH SOLVENTS.

Finally, turpentine may be recovered from wood by dissolving it in a volatile or nonvolatile solvent, or in an alkali. Extraction with volatile solvents is but little employed, and deserves more consideration than it has received. The economical working of solvent processes is dependent primarily on the possibility of a practically complete recovery, at a minimum cost, of the large quantities of solvent which are required to saturate the wood for extraction. Fortunately the solvent can be almost entirely recovered. The products obtained by the use of volatile solvents of low boiling point are of high quality; the rosin is clean and bright, while the turpentine is comparatively free from materials produced in the extraction.

This method has been studied by this bureau and by other investigators, and is perfectly simple and feasible, but for the reasons mentioned it remained undeveloped until very recently. It is now being placed on a commercial basis.

The following diagram shows the products obtained from resinous wood by extracting with volatile solvents and the utilization of the extracted chips:

Resinous wood (hogged and extracted with volatile solvent)	Extract (distilled)	Distillate (fractionated)	Solvent. Wood turpentine.
		Residue (strained)—rosin.	
	Chips	Destructively distilled (see p. 10.)	
		Wasted or burned.	
		Digested with caustic soda—paper pulp.	

Several processes have been developed in which liquids of higher boiling points than turpentine are used. Rosin, pitch, tar, pine oil, or gum turpentine is suitable for starting the plant. When treating wood rich in resins the bath finally consists of rosin and its decomposition products. With lean wood the bath must be added to from time to time. The liquid passes through a superheater, where its temperature is raised to about 200° C., and thence to a closed retort containing the wood so prepared as to insure rapid penetration by the liquid. In returning to the superheater the hot rosin passes through a still, and the turpentine is distilled from it. The liquid is finally drained from the exhausted wood and used on subsequent charges. The advantages of this process over simple distillation with steam are that the treated wood is left in such condition that it may be sold for fuel, used as paving blocks, or subsequently destructively distilled without contaminating the wood turpentine with rosin spirits or other products of destructive distillation.

The recovery of turpentine and rosin by extraction with alkalis and the use of the extracted wood for making paper is a process which has been experimented with, and one mill is operating on mill waste, making turpentine and wrapping paper. In this process also the wood is cut up in the same way as for making paper pulp and treated with an excess of soda in solution. The turpentine may be removed by simple steaming previous to adding the soda solution, or it may be steamed off during the cooking with soda. The latter procedure has the advantage of requiring less time to complete the treatment of the wood. The cooking with soda may be conducted in several ways. The contents of the retort may be heated only sufficiently to dissolve the rosin without attacking the wood, after which the solution may be drawn off nearly free from ligneous materials, and the rosin or rosin oils recovered from it, or the wood may be cooked with the soda solution at a high temperature.

After the completion of the treatment the soap solution of soda and rosin, containing also the dissolved ligneous material, is drained and washed out of the pulp, which, after suitable treatment, may be disposed of in several ways. The removal of the turpentine by this process is exceedingly rapid, and it is claimed that from the boiling alkali solution the turpentine may be removed by some processes in from 10 to 15 minutes.

REFINING WOOD TURPENTINE AND WOOD OILS.

The crude oil obtained by any distillation process is redistilled or refined before it is marketed. Crude oils from the destructive process are agitated with alkali whereby the greater part of the phenols, cresols, and related bodies are dissolved, and separated by settling from the turpentine, rosin spirits, and rosin oils, which are run off and separated by redistillation with steam as steam-distilled wood turpentine is. This is usually done in a copper-pot still of suitable size, live steam being conducted directly into the crude oil. Ordinarily this distillation is not conducted with care, and but little attempt is made to insure that the wood turpentine obtained does not contain considerable quantities of the heavier "pine oils." Distillation is usually continued until the oil passing over shows "bead" or until the specific gravity is about 0.8800 or 0.9000 at 15.5° C.

Recently at a number of plants more care has been exercised in refining and the product is distilled with steam several times from a pot still. Such careful refining is, of course, expensive and the yield is much reduced, but the product complies closely in specific gravity and behavior on distillation and evaporation with standard specifications for turpentine. The crude oil yields on redistillation, as this is commonly practiced, from 60 to 80 per cent of wood turpentine. As will be seen from the analyses (Table 1, p. 58), these oils contain notable quantities of heavier oils, and but few of them can be considered high-grade wood turpentine.

The pine oils from steam distillation are also redistilled with steam and are marketed in two grades, white and yellow or straw colored. Their specific gravity ranges from 0.8890 to 0.9600; refractive index from 1.475 to 1.500 and they begin to distill at from 165° to 180° C. Distillation is generally complete at from 215° to 240° C. and by far the larger quantity of most pine oils distills between 190° and 225°. The crude, destructively distilled oils, after being agitated with alkali and washed with water to remove phenols, cresols, etc., are separated into at least three fractions, preferably in a column still. The first fraction, distilling at from 80° to 150° C., consists essentially of the lighter constituents of rosin spirits with some turpentine and is contaminated with small portions of substances derived from the breaking up of the wood. The fraction boiling at from 150° to 180° C. is destructively distilled wood turpentine, that boiling at from 170° to 180° C. to 230° to 250° C. constitutes the pine oils with the heavier constituents of rosin spirits, and the fractions boiling at from 250° to 400° C. consist of rosin oils contaminated with decomposition products from the wood substance.

The alkali solution after separation from the crude oils contains the wood creosote, tar, and other alkali-soluble bodies. The wood creosote and tar acids may be separated by adding sulphuric acid in excess, washing the separated material several times with water, and further refining, if desired, by distillation. (See Table 2, p. 60, for analyses of pine oils and still residues.)

EQUIPMENT FOR THE MANUFACTURE OF WOOD TURPENTINE.

The equipment of a plant for the destructive distillation of resinous woods would, of course, vary with its capacity, size and shape of retort, and arrangement of the plant. Small retorts of about 1 cord capacity are most commonly used, and an approximate idea of the kind and quantity of apparatus required for a plant distilling 12 cords per 24 hours is given by the following list:

12 retorts, 1 cord each.
12 condensers.
100 100-pound charcoal cans.
2 tar stills.
1 turpentine refining still.
2 or 3 settling vats for crude turpentine, crude pyroligneous acid, and free tar.
1 100-horsepower boiler.
1 20-horsepower engine.
Pumps for supplying water to condenser and handling turpentine, etc.
Storage tanks for crude and refined products.
Conveyers and copper piping.
Suitable buildings for housing equipment.

The yield of wood alcohol and acetic acid is less than half of that obtained from hard woods, or about 4 gallons of alcohol and from 50 to 90 pounds of acetate of lime, and practically no effort is made to recover either of these products. In case these are recovered, additional stills, tanks, and steam pans are required.

The cost of such a plant varies greatly in accordance with the quality and completeness of the equipment and with local labor and other conditions. Reliable builders of such plants quote from $1,000 to $2,000 per day cord on the basis of a 10-cord plant, with proportionately higher prices for smaller plants and lower prices for larger plants. For the steam distillation of wood where only turpentine and pine oils are recovered, a less elaborate equipment is required. For a plant of 10 units, the following list gives an idea of the equipment needed:

10 retorts, 1 cord each.
10 condensers.
2 150-horsepower boilers.
1 100-horsepower engine.

1 refining still and condenser.
2 hogs for chipping wood (1 in reserve).
Storage tanks for crude and refined turpentine.
Pumps and piping for water supply and turpentine.
Conveyers.

The cost of such a plant will vary from $10,000 to $35,000, depending largely on the value placed on patent rights (many forms of retorts and other apparatus are patented, and as a rule the right to use is charged in the cost of the plant), and on the number of charges the plant will handle in 24 hours. The cost of a plant to take from four to six charges in 24 hours is, of course, greater than that of the same plant built to handle but one charge in the same time, though the cost on the basis of the number of cords of wood handled daily may be much less.

These lists of apparatus and estimates of cost of plant are simply intended to give a general idea of the equipment formerly furnished by builders and now in common use in the older plants, together with its approximate cost. No attempt is made to give details in regard to the machinery, arrangement, or housing. The boiler, engine, and condenser capacity of these older plants was rarely sufficient for maximum efficiency. Other special equipment now employed at several plants and considered preferable is described on page 54. The full equipment and its actual cost can be determined only when the location of the plant and the kind and quantity of work required are known.

COST OF PRODUCING WOOD TURPENTINE.

The cost of producing wood turpentine varies greatly in different plants and is dependent on the cost and quality of the wood obtainable for the distillation, the cost of operating and managing the plant, depreciation of the plant, and interest on the investment. The cost of wood, according to information collected by the bureau, varies from $1.50 to $3 per cord for what is known as "lightwood," delivered at the works. This charge is made up chiefly of the cost of gathering, hauling, and freight, the wood in the forest costing very little—from 15 cents to 75 cents per cord being a fair valuation. While it is the aim of the distiller to use only wood rich in turpentine and rosin, it is often quite difficult to get this, particularly when the wood is delivered at the works or at the railroad on contract, as the contractor does not as a rule exercise much care in selecting the best and richest wood. Better wood is undoubtedly obtained when it is selected and gathered by the distiller's own force, and, furthermore, the cost of hauling and handling is not so great as a rule as when it is done by contract. The cost of waste wood from a lumber mill averages much less, probably not more than $1.50 per cord.

The cost of operating destructive distilling plants, including office expenses, labor, fuel, and packing products, is variously estimated by the owners and exploiters at from $2.50 to $6 per cord, while the charges for interest and the depreciation of plants are figured at from 60 cents to $1.60 per cord of wood distilled. The cost of operating a steam distilling plant is estimated at from 47 cents to $1.82 per cord and the charges for interest and depreciation are from 24 cents to 61 cents per cord. The cost of operating plants may be summarized as follows:

Relative cost of operating steam and destructive distillation plants.

Items.	Destructive distillation, per cord.	Steam distillation, per cord.
Cost of wood for distilling	$1.50 to $3.00	$1.00 to $3.00
Management, labor, fuel, packing	2.50 to 6.00	.47 to 1.82
Interest and depreciation	.60 to 1.60	.24 to .61
Total	4.60 to 10.60	1.71 to 5.43

It must be borne in mind that in destructive distillation other products, such as charcoal, pine oils, and rosin oils, are produced, the value of which, under proper conditions, should more than cover the greater cost of operating the destructive process.

The cost figures given vary so widely that it is quite evident that they may be greatly reduced by proper equipment and more rapid handling of the material. This statement applies particularly to the steam processes, in which this laboratory has found that the average time of distillation may be greatly reduced without lowering yields. Indeed, the greatest field for improving the wood-turpentine industry lies more in reduced cost of production than in increasing the yields per cord of wood, though there are, of course, great possibilities here also. In the examinations which have been made of wood-turpentine plants in the South, one is particularly impressed with the crudeness of equipment and the wasteful labor conditions which prevail in most cases. Even in the most modern plants decided improvements in arrangement and operation could be made. The possibilities of increasing profits by improvements in these particulars seem to be exceedingly inviting. Necessarily, improvement along these lines can only be made by competent management, and all such plants should be under the control of or have the advice of an able chemical engineer. The cost of production may also be largely reduced by a proper location and affiliation of the plant.

YIELDS OF VARIOUS PRODUCTS FROM THE DISTILLATION OF RESINOUS WOODS.

The yield of crude oil obtained is governed by two factors—the quantity of oil in the wood and the completeness with which this is removed by the method used. Thus, by steam processes from 2 to 15 gallons per cord are recovered from sawdust and slabs, from 8 to 20 gallons from lightwood, and as high as 30 gallons from very rich lightwood. By destructive processes from 10 to 20 gallons are recovered from ordinary lightwood, and larger quantities from exceptionally rich lightwood. By extracting with soda it is claimed that somewhat better results are obtained.

The operators claim that when using fixed retorts and the steam process a good yield from lightwood, under usual conditions, is approximately 15 gallons of crude oils per cord; with rotary retorts the production is increased to about 18 gallons, and experiments have shown that at least 3 gallons of crude oil, containing about 25 per cent of actual turpentine, are often left in the wood. The crude oil yields about 80 per cent of refined oil—a result which is only obtained by distilling much of the heavy oil with the turpentine. By the destructive process the yield of wood turpentine is several gallons per cord greater than by the steam process.

The data on yields of various products as summarized in the following tables were obtained from many sources, mostly from wood distillers, some from the literature, and some based on the experimental work of the bureau. The whole is harmonized as far as possible in accordance with the experience and judgment of the author. The data on yields of crude oils, refined wood turpentine, charcoal, methyl alcohol, acetate of lime, and unbleached wood pulp may safely be regarded as quite accurate. In fact, all the data on the steam and volatile solvent processes may be so regarded. The other data on pine oils, rosin spirits, rosin oils, and creosote, by the destructive and alkali extraction processes, are not exact and in general are to be regarded as careful estimates rather than as known results. This is due to the fact that less attention has been paid to the separation and utilization of these products, and what one producer terms "creosote" may in reality embrace all oils heavier than pine oil, or, in some cases, those heavier than turpentine. In other plants all oils heavier than pine oils may be combined with the tars and disposed of under this term. Further work will be necessary before more definite information can be given as to the quality and nature of the products variously termed "rosin spirits," "rosin oils," "creosote," "wood oils," "tars," etc., obtained in the destructive distillation of pine wood.

Data on the yield of various products from 1 cord (4,000 pounds) of long-leaf yellow pine ("lightwood").

Products.	Steam process.		Destructive process.		Alkali extraction.		Volatile solvents.	
	Distills between—	Yield.	Distills between—	Yield.	Distills between—	Yield.	Distills between—	Yield.
	°C.	*Gallons.*	*°C.*	*Gallons.*	*°C.*	*Gallons.*	*°C.*	*Gallons.*
Crude oil	154-250	8-20	80-400	36-120	154-400	36-120	154-250	8-20
Refined wood turpentine	150-180	6-16	150-180	5-20	150-180	5-20	150-180	6-16
Pine oils	175-250	2-4	175-250	2-5	175-250	2-5	175-250	2-4
Rosin spirits			80-150 180-250	2-10	80-250	5-15		
Rosin oil			250-400	20-65	250-400	20-60		
Creosote			100-400	8-20	100-400	8-20		
Rosin								[1] 300-800
Methyl alcohol				½-4		½-4		
Calcium acetate				[1] 40-80		[1] 5-15		
Charcoal				[2] 30-50		[2] 10-25		
Paper pulp, unbleached		[1] 1,000-1,600				[1] 1,000-1,600		[1] 1,000-1,600

[1] Pounds. [2] Bushels.

Approximate yields of various products from 1 cord (4,000 pounds) of lean long-leaf yellow pine.

Products.	Steam process.	Destructive process.	Alkali extraction.	Volatile solvents.
	Gallons.	*Gallons.*	*Gallons.*	*Gallons.*
Crude oils	2 -10	20-52	20 -50	2 -10
Refined turpentine	1½-8	2 -10	2 -8	1½-8
Pine oils	½-2	½-2	½-2	½-2
Rosin spirits		2 -5	3 -8	
Rosin oils		12 -27	10 -25	
Creosote		4 -8	4 -8	
Rosin				[1] 75 -400
Methyl alcohol		½-5	½-5	
Calcium acetate		[1] 40 -100	[1] 5 -15	
Charcoal		[2] 35 -50	[2] 10 -25	
Paper pulp, unbleached	[1] 1,000 -1,600		[1] 1,000 -1,600	[1] 1,000 -1,600

[1] Pounds. [2] Bushels.

In these tables the distillation is assumed to be carried to completion in all cases. The tar which is formed is distilled, yielding rosin spirits, rosin oils, and creosote. Therefore tar is not given as one of the distillation products of wood, inasmuch as it is only an intermediate product. In practice the impure heavy oils which run out of the wood or result from the partial breaking up of the wood collect on the floor of the retorts and are withdrawn before they are completely distilled by the rising temperature of the retort. Tars from resinous woods consist, therefore, of the phenoloid bodies, cresols, and other related bodies from the incomplete distillation of the wood substance, mixed with pine oils, rosin spirits, rosin oils, rosin, and small quantities of organic acids. The quantity of tar per cord is somewhat less than the sum of the rosin spirits, rosin oil, and creosote. The maximum yield will rarely equal the highest figures given for those products. The "rosin spirits" and "rosin oils" obtained by destructively distilling pine or its soda extract are necessarily contaminated, as is also the turpentine, with empyreumatic constituents derived from the distillation of the cellulose and lignin. They are not, therefore, strictly speaking, "rosin oils" and "rosin spirits," but may properly be termed "wood-rosin spirits" and "wood-rosin oils."

These facts should be borne in mind in considering the tabulated data on yields. It is hoped that more definite data as to the yield of various products from pine will soon be available. The operations in the past at wood turpentine plants have as a rule not been conducted with the care necessary to determine even average yields with exactness. The weight of a cord of wood varies greatly, and the amount of water in the wood may also vary. In order that exact data may be had, the wood at turpentine plants should be weighed, a sample analyzed, and all products measured or weighed. The wide values given for the several products are explained partly by variations in the composition of the wood; as has been stated, pine wood differs greatly in the quantity of resin it contains.

The following figures are taken from the census reports:

Statistics on the production of wood turpentine and other resin oils from wood (census reports).

Years.	Wood used.		Turpentine produced.			Oils produced.	
	Cords.	Value.	Gallons.	Gallons per cord.	Value.	Gallons.	Value.
1906	50,000	$129,000	503,000	10.1	$239,000	125,000	$17,000
1907	62,000	211,000	655,000	10.5	305,000	392,000	69,000
1908	99,000	202,000	506,000	5.1	166,000	305,000	56,000
1909	116,000	234,000	683,000	5.9	243,000	323,000	70,000

Though the quantity of wood used in this industry has more than doubled in five years, the quantity of turpentine reported shows some fluctuation and a rather small increase. Owing to the fact that the products of both steam and destructive processes are included in the summary, the only conclusion warranted seems to be that the yield of turpentine per cord has greatly decreased, and this decrease is not balanced by an equivalent increase in the reported heavy oils. It seems probable that the decreased yield per cord reported in 1908 and 1909 is partly accounted for by greater care in refining, whereby the heavy oils are more completely separated. The decreasing yields indicate either that the wood used in late years is not so rich as that formerly employed, or that the distillation is less complete, or that a different system of reporting is employed.

PROPERTIES AND COMPOSITION OF WOOD TURPENTINE.

Ordinary gum turpentine recently prepared by distilling the crude gum may be defined as a mixture of terpenes having the same empirical chemical formula, $C_{10}H_{16}$, together with small quantities of certain derivatives of these terpenes, concerning which but little is known. These components differ in specific gravity, optical properties, boiling point, consistency, refractive index, odor, and probably in other physical and chemical properties. The peculiar suitability of gum turpentine as a diluent for paints and varnishes is due apparently to the fact that it evaporates neither too rapidly nor too slowly—not so fast as to leave the coated surface full of fine pores through which moisture may enter and destroy the paint or varnish, nor so slowly as to unduly prolong the drying. Further, its oxygen-carrying power hastens the oxidation of the linseed oil, and thus forms the binding coat which holds the paint bases and resins to the surface of the material.

Crude wood turpentine differs from gum spirits primarily in that it contains additional terpenes and terpene derivatives, together with other nonterpene derivatives. In that obtained by the destructive distillation of long-leaf yellow pine, dipentene, pentane, pentene, toluol, heptine, etc., have been reported, in addition to pinene.

After washing with soda to remove phenoloid bodies derived from the breaking up of the wood, fractional distillation, or fractionation alone in the case of the steamed distilled oils, will separate these mixtures into two or more portions, each of which will consist very largely of constituents whose boiling points are very close together. It is impossible, however, by distillation to separate those compounds having nearly the same boiling points, and as a matter of fact each fraction will also contain more or less of the heavier oils, depending on how carefully the fractionation has been conducted. Thus, from the destructively distilled oils, fractions distilling at from 80° to 155° C., from 155° to 180°, from 180° to 220°, from 220° to 250°, from 250° to 400°, or, as a matter of fact, between any desired limits, may be obtained.

The first fraction distilling between 80° and 154° C. closely resembles rosin spirits, and is in fact the lighter portion of rosin spirits contaminated with small quantities of materials from the nonresinous wood constituents. The portion passing over between 154° and 180° C. is destructively distilled wood turpentine. This fraction contains pinene and dipentene, together with smaller quantities of other compounds, at least some of which are normal constituents of rosin spirits. The heavier oils distilling from 180° C. up are pine and rosin oils in indefinite mixtures. They are used in the manufacture of lubricants, printing inks, solvents, etc. The crude oils and tars of the destructive process are often used as disinfectants, cable coatings, wood preservatives, shingle stains, etc.

The crude oils obtained by the steam distillation of long-leaf yellow pine contain pinene, camphene, limonene, dipentene, terpineol, borneol, fenchyl alcohol, camphor, cineol or eucalyptol, etc. They are fractionated to two or three fractions, wood turpentine distilling at from 150°–160° to 175°–180° C., light pine oils between 170°–180° and 210°–225° C., and heavy pine oils at from 180°–190° to 230°–240° C.

Steam-distilled wood turpentine consists essentially of pinene, together with camphene, limonene, dipentene, cineol, and, depending on care of fractionating, more or less, terpineol, borneol, terpinene, etc.

The pine oils contain chiefly terpineol, borneol, and fenchyl alcohol, with small quantities of limonene, dipentene, terpinene, cineol, and even less pinene and camphene. Pine oils are used in medicine, in the manufacture of artificial camphor, terpineol, and terpene hydrate, as a solvent for pyroxylin in cheap varnishes and in other varnishes.

Many efforts have been made to remove from wood turpentine one of its distinguishing characteristics, namely, its peculiar odor, but so far with only partial success, as the odor often returns. Our own experiments indicate that this odor is due chiefly, if not entirely, to the heavy oils which wood turpentine usually contains. When these are removed by careful fractionation, wood turpentine consists almost wholly of the same constituents as gum spirits, which it also closely resembles in odor.

The first fractions obtained from crude steam-distilled turpentine resemble gum spirits very closely in odor and composition, but the yield of this portion is too small to justify the rejection of subsequent fractions. Hence, in separating from the crude oils all those that have approximately the same specific gravity and behave like turpentine when distilled, portions of the before-mentioned heavier oils distill and mix with the light oils and it is probably the presence of these heavy oils which accounts for the difference between gum spirits and wood turpentine. Destructively distilled turpentine with which the products of the distillation of rosin are mixed can not be freed entirely from rosin spirits and its accompanying odor. The following table shows the differences existing between commercial gum and wood turpentines in regard to those properties by which technical value is usually judged. The composition of wood turpentine is also shown by the analyses given in Table 1, page 58.

Constants of commercial gum and wood turpentines.

Determinations.	Gum spirits.	Steam distilled.	Destructively distilled.
Specific gravity at 20° C.	0. 8617 to 0. 8889	0. 859 to 0. 9150	0. 857 to 0. 898
Angular rotation at 20° C. in 100 mm tube	−34. 8 to +29. 6	+16. 5 to +36. 14	+34. 4 to +77. 6
Index of refraction at 20° C.	1. 4684 to 1. 4818	1. 4673 to 1. 4755	1. 4666 to 1. 4810
Initial distilling point [1]	154 to 159° C.	153 to 177° C.	150 to 169° C.
Distilling below 170° C. per cent	73 to 99	0 to 95	0 to 93
Distilling below 185° C. per cent	88 to 99	20 to 98	61 to 97
Iodin absorption [2]	350 to 400	300 to 362	300 to 398
Acid number	0. 140 to 0. 286	0. 080 to 0. 312	0. 028 to 0. 246
Saponification number	2. 44 to 8. 60	1. 06 to 8. 75	0. 65 to 4. 32
Color (Lovibond):			
Yellow	0. 7 to 2. 5	0. 5 to 10. 0	0. 4 to 4. 5
Red	0. 0 to 0. 5	0. 2 to 1. 4	0. 0 to 0. 8

[1] Emergent stem thermometer; uncorrected.
[2] Wjiss solution. Stood one-half hour; 200 per cent excess iodin.

From these figures it is quite evident that both steam-distilled and destructively distilled turpentines have many of the same general characteristics as gum spirits. A point of considerable interest, in view of the often-repeated complaint of painters that wood turpentine is strongly acid and because of this is very trying to the eyes, is the comparative acid numbers of the several classes of turpentine. There is no evidence to indicate that the acidity of wood turpentine is greater than that of gum spirits, though of course the acids may not be the same. The acid and saponification numbers are indicative of the amount of oxidation products which are present. These figures are not so striking, however, as is the iodin number discussed below. While it is true that the limits of specific gravity and of the quantity distilling below 170° C. do not differ widely among these three classes of turpentines, it is also true that the specific gravity will average higher and the quantity distilling below 170° C. lower in wood turpentine than in gum turpentine. In other words, the wood turpentine now on the market does contain too much pine oil distilling above 170° C., and to this fact, chiefly, is undoubtedly due the dissatisfaction which is expressed with reference to its drying properties.

The iodin number also throws light on the composition of the turpentines. It indicates that in certain cases the oil contains large quantities of constituents other than pinene or other terpenes of the same formula. In other words, the iodin number indicates practically what a distillation does—that is, the approximate quantity of heavy oils present. It will be seen from Table 2 that, compared with good gum spirits or with the light oils from crude wood turpentine, the heavy oils have a low iodin number; the iodin number therefore shows whether or not the sample contains considerable quantities of heavy oils, and also that these heavy oils are not of the same nature as the light oils. The same fact is shown in a general way by the saponification number. Those turpentines which have large residues that do not distill below 185° C. have high saponification numbers, or combine with much soda. This saponification is roughly proportional to the residue not distilling below 185° C.

The results on the crude oils (Table 3) differ from those on the refined products only in degree. The crude oils have somewhat higher specific gravities, absorb less iodin, and smaller proportions distill below 170° C., while the color is deeper than in the refined oils. The figures on crude oils show that as a rule they contain but little more heavy oils than the refined samples. In other words, but little has been accomplished in improving the product except by lightening the color. This is additional evidence that not enough care is exercised in refining.

PINE OILS.

"Pine oil" is the term applied to the heavier oils contained in crude wood turpentine. The term is more properly limited to the heavy oils of the steam-distilled crude oil. The heavy oils of crude destructively distilled wood turpentine are chiefly the heavier portions of rosin spirits and rosin oils.

Until very recently pine oil was almost entirely a waste product and the producer had some trouble in disposing of it. It is now finding more extensive use in paints, varnishes, and medicine, and in the manufacture of drugs and chemicals. Analyses of these oils, crude and refined, and also of refining still residues are given in Table 2, page 60. The samples which have been examined in this laboratory have specific gravities of from 0.889 to 0.944, a refractive index varying from 1.4765 to 1.4855; the initial distilling turpentine, when water is not present, ranges from 170° to 210° C. and the major portion generally distills between 200° and 215° C. The iodin values are much lower than those of the refined wood turpentine, while the saponification numbers vary about like those of gum spirits.

PRINCIPLES OF FRACTIONAL DISTILLATION.[1]

When a mixture of liquids is distilled the vapors which arise always contain a larger proportion of the lighter or low boiling constituents and a smaller proportion of the higher boiling constituents than does the remaining liquid, but rarely, if ever, under commercial conditions, do they consist of these lower boiling constituents alone. The composition of the vapors which arise from the liquid bears a very definite relation to the composition of the liquid in the still. The greater the proportion of the heavy constituents in the liquid, the greater their proportion in the vapor. When an ordinary pot still, therefore, is employed even the first portion which passes over carries with it more or less of the higher boiling constituents. The proportion of these heavier constituents thus carried over is primarily controlled by the proportion in the liquid. It is also greater the less the fractionating effect in the still. Fractionation, within limits, is decreased by increasing the velocity of the distilling vapors; that is, the more rapid the distillation, the smaller the space above the liquid in the still, and the smaller the opening to the condenser, the more of the heavy constituents the distillate contains. As distillation proceeds, the liquid in the still becomes richer in the heavier constituents and the vapors arising from this liquid also contain a larger proportion of these, although less than the remaining liquid, until finally all of the lighter constituents are removed and the distillate consists wholly of the heavier ones. That is, in the distillation of a crude turpentine from a pot still, the first part of the distillate is a fairly light product, containing relatively small proportions of heavy oils. This first portion is followed sooner or later, depending on the proportion of light oils in the crude oil and the speed of distillation, by a product containing continually increasing proportions of heavy oils, until at a certain point the product no longer contains light oils but consists entirely of what may be called the light pine oils mixed with small proportions of the heavy ones. As distillation progresses this product also gradually changes in composition and the proportion of heavy pine oils becomes greater until they form the entire distillate. For these reasons it is impossible to secure more than an approximate separation of crude wood turpentine into its several chief constituents by a single distillation from a pot still. It is necessary to make several redistillations of each of the intermediate fractions or portions which have been distilled between certain limits. Directions for doing this most satisfactorily are given on page 55.

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[1] For extensive discussions of the subject of distillation see Hausbrand, "Evaporating, Condensing and Cooling Apparatus;" Rechenberg, "Gewinnung und Trennung der aetherischen Oele;" and Young, "Fractional Distillation."

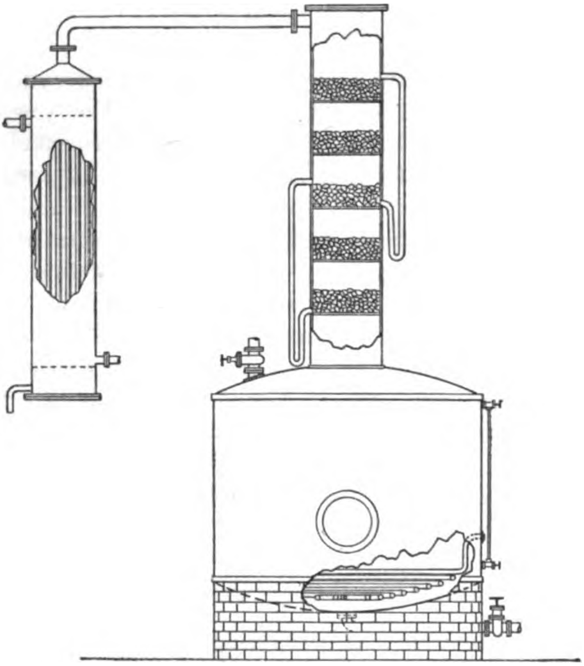

FIG. 1.—A simple periodic column still, with chambers containing broken rock.

The separation of the several constituents of a mixed liquid is most quickly, economically, and completely effected in a column still, several common forms of which are shown in figures 1, 2, 3, and 4. This consists essentially of a number of small, simple stills placed one above the other in the same column. The general statements made with reference to the liquid and vapor in a simple still are equally true of the contents of each still or chamber of the column still. The vapors above the liquid in the lowest chamber are richer in the lighter constituents than is the liquid itself. These vapors pass upward through perforated plates or capped openings (see figs. 3 and 4) and, partially condensing, become the liquid in the second chamber, and the vapors in this chamber are in turn richer in the lighter constituents than is the liquid in the same chamber. This relation is repeated in each successive chamber until the vapors at the top of the column, in the earlier stages of the distillation, are practically pure or constitute a mixture of definite composition. If, when this point is reached, the heavier constituents of the liquid are not removed from the still, but the distillation is continued with rising temperature, these constituents pass upward into each successive chamber, obeying the same law as in distillation from the simple still. As there are a number of successive stills, however, the mixed liquid when passed through a column still once is, as a rule, separated more completely into its several constituents than by several distillations in a pot still.

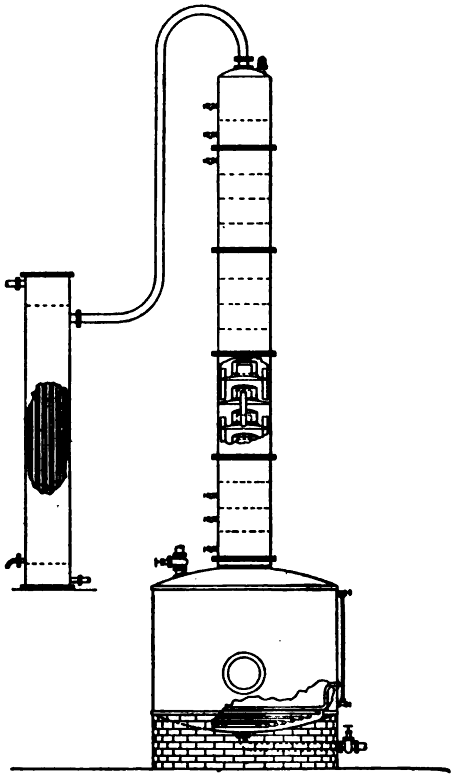

FIG. 2.—A periodic column still, with the chambers separated by hooded plates.

Several forms of column stills applicable to the refining of wood turpentine will be considered. Figure 1 shows one of the simplest forms. It consists of the still proper, surmounted by a fractionating column. The column consists of one or more chambers, containing broken quartz rock or other inert material, or of a number of chambers separated by plates, as shown in figure 2. The former is the cheaper, but the latter is somewhat more efficient. Stills of the type shown in figures 1 and 2 are known as periodic; that is, they are not operated continuously, but distillation is discontinued from time to time, the residues emptied out, and the still recharged for another distillation. By careful and intelligent operation crude wood turpentine can be separated into three or four products of fairly definite proportions, each one passing in succession through the column and being condensed and stored in the proper receiving tank. It is preferable, however, to distill the wood turpentine through the column and the pine oils from a pot still, to which they are run from the bottom of the column still. Pine oils distill so slowly with steam that the use of a fractionating column in their case is not practicable. Periodic column stills are suitable only for small plants producing less than 600 or 800 gallons of crude oils per day, in which case the volume of the products does not warrant the installation of the more costly continuous still for the separation of three or more products simultaneously.

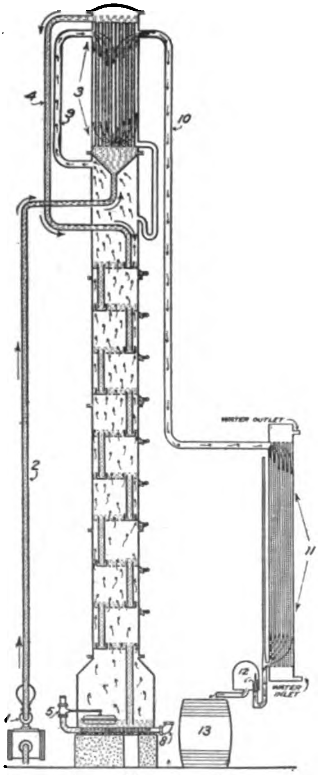

FIG. 3.—An ordinary continuous beer still used for turpentine distillation.

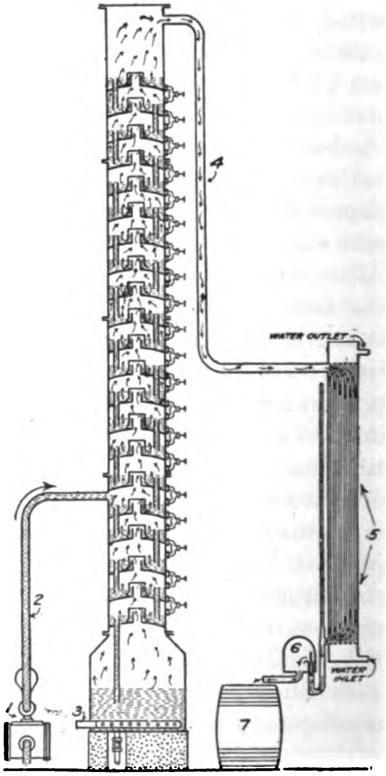

FIG. 4.—A continuous still with hooded plates.

Two forms of the continuous still are shown in figures 3 and 4. These may be operated together to obtain three products, or individually to obtain two products. The crude turpentine enters the column through pipe 2 in a continuous stream. Here it comes in contact with live steam entering below and is carried upward where it is fractionated; the light oils of any desired composition, depending on the volume, speed, and temperature of the entering steam, pass continuously from the head of the column, are condensed, and run into storage tanks, while the heavier oils pass downward and out at the bottom of the still into a second and usually smaller column, or into a pot still from which they are again distilled. This distillate is pine oil of one or more grades and is heavier than that obtained from the first column. The condensed water and small quantities of other materials appear as waste at the bottom of the shorter column or remain in the pot still. The distillate from the first column should, under proper conditions of operation, contain all the wood turpentine and but little of the heavier constituents, such as the pine oils, etc., of the crude oils. These pine oils, if present, can be separated by a second distillation of the portion containing them. The composition of the first distillate may be largely controlled by the manipulation of the still, especially by the speed and the ratio between the steam and the crude oils entering the still. The pot still may be readily converted into a column periodic still by replacing the gooseneck with a suitable column.

It is highly important in turpentine distillation that the condensing surface be ample to fully condense the vapors and cool the condensed liquids to about 70° F. An insufficient condensing surface is too often a fault of wood distillation plants. It is to be borne in mind that in the South the water available for condensing and cooling has a higher temperature than that used farther north, and consequently larger condensers and more water are required. The consumption of cooling water may be greatly reduced by the use of large atmospheric condensers.

EXPERIMENTS IN REFINING WOOD TURPENTINE.

The purpose of refining crude wood oils is to separate them into their constituents or into fairly definite mixtures of commercial value. That portion known as "wood turpentine" should be similar to freshly distilled gum spirits as a paint and varnish thinner and be as free as possible from the very objectionable odor of the crude or imperfectly separated wood turpentine. Properly made wood turpentine should dissolve gums and resins as well as gum spirits, and when used in paints and varnishes they should be as durable and dry rapidly without pinholes and without crawling.

Further, it should be no more deleterious to the health of the workman than the gum spirits.

The refining experiments conducted in this laboratory have been controlled by determining the specific gravity, refractive index, initial boiling point, and behavior on distillation of samples drawn at stated times during the refining operations and of the several final products obtained from these operations.

USE OF THE PERIODIC COLUMN STILL.

The refining experiments on an industrial scale have been made with pot stills and two forms of periodic columns, which are shown in sections in figures 3 and 4. Figure 3 is an ordinary beer still of 10 chambers, such as is used in making alcohol, modified and adapted to the work. Both columns were used as periodic stills. The steam necessary to boil the crude turpentine is admitted to the bottom chamber of the column through the pipe 8. It boils the crude oils in this chamber and passes upward through one chamber after another, boiling the descending crude oil and carrying with it the turpentine vapors. The plates within the column are perforated so as to allow the steam to pass through them. The vapors rise to the upper part of this column and are conveyed through the pipe 9 into the heater 3, where they are circulated about the tubes containing the crude oil passing through the heater, and then are carried through the pipe 10 into the condenser 11 and thence to the separator 13 through the test box 12.

The crude turpentine is discharged from pump 1 and pipe 2 into the heater 3. This heater contains a series of tubes through which the turpentine is passed and around which the vapors coming from the boiling oils in the column on their way to the condenser are circulated. The heated turpentine leaves the heater through pipe 4 and reenters the column below the heater where it comes in contact with steam. The lower part of the column is divided by plates into a series of chambers, in each of which the turpentine is boiled and relieved of some of its light oils. The crude oil takes a downward course through the drop pipes and across each of the various plates. It should lose all of its light oils by the time it reaches the bottom chamber of the column, from which it is automatically discharged through valve 5, or subsequently distilled as pine oil, after the turpentine has been removed.

Figure 4 was also used as a periodic still in practically the same way. The crude oils should flow from a storage tank into the still through pipe 2. The column contains a series of chambers, upon each of which is carried about 3 inches of liquid. A constant level is maintained in each chamber by means of the drop pipes. The plates between the chambers are not perforated as in figure 3, but the vapors ascend from one chamber to another through a pipe in the center of the plate, are deflected downward by a hood over the pipe, and forced to boil their way through the liquid on each plate by the steam which enters the still through the pipe 3. The arrows indicate the course of the vapor in the still. The vapors boil from chamber to chamber, becoming purer as they ascend until they reach the top chamber of the column, from which they are delivered through the pipe 4 into the cooler condenser 5, where they are reduced to a liquid. This cooling and condensing is effected by circulating cold water through the tubes around which the vapor passes.

The condensed vapor, turpentine, and water is drawn off at the bottom of the condenser and allowed to flow through the test box 6, where it can be examined. The steam pressure within the apparatus is registered by a pressure gauge not shown. The turpentine and water after passing through the test box is run into the separator 7, from which the turpentine may be run to storage tanks.

The general plan of the experiment was to draw off samples from time to time during the distillation, noting the temperature of the vapors escaping to the condenser and the relative volumes of turpentine and water in the condensed distillate, and making an examination of the samples, determining specific gravity, refractive index, initial distilling temperature, and behavior on distillation when 150 cc were distilled from an ordinary distilling flask, using an emergent stem thermometer. All of the turpentine obtained during a distillation was not run into the same tank, but at various predetermined periods it was directed into other tanks, and at the close of a distillation each fraction was measured, and the relative volume of water and turpentine determined. The residue of heavy oil was also measured, and samples from each tank were examined as just outlined. The results of the distillation and data obtained on the distilling oils from time to time during the process are given in Tables 4 and 5.

These data show the effectiveness of the separations at intervals during distillation and are in all respects similar to data obtained in the distillation of other closely related oils with steam in a column still. The first portions distilling consist almost entirely of light oil, but as distillation proceeds the proportion of light oil in the distilling oils decreases while the proportion of heavy oil increases until distillation is discontinued. The fact that the proportion of heavy oil begins to increase when the distillation is about half over is proof that large quantities of heavy oil were in the crude product. The first or A fractions of all distillations in the beer still were mixed together, and the A fractions of all distillations from the refining still were mixed together; each mixture was then again passed through the still previously used. The data in Table 7 show that a second distillation gave a refined wood turpentine which complied with the most exacting requirements as to uniformity, specific gravity, and behavior on distillation; furthermore, the odor of this turpentine is much milder than that of the original crude oils, resembling quite closely that of gum spirits.

Table 6 contains all of the complete results obtained on the samples collected in the experiments in refining crude steam-distilled wood turpentine. The table is arranged on the basis of the specific gravity of the sample, that with the lowest gravity being entered first. From this table the successive samples of any distillation may be readily picked out. For instance $6A_1$ and $6A_2$ are the successive samples of the first fraction of the distillation of the sixth barrel and $9B_3$ and $9B_4$ are the successive samples from the second or B fraction of the distillation of the ninth barrel.

From the nature of the experiments, all of the data can not be strictly harmonious. Variations in atmospheric pressure occasion differences in distilling temperatures. Variations in steam pressure and volume occasion differences in distilling temperatures, and within limits in specific gravity and behavior on redistillation. On the whole, however, the data (see Tables 4 to 8) show that a low distilling temperature, specific gravity, and refractive index, together with a high percentage of turpentine in the steam distillate, are characteristic of turpentines of which 90 per cent distill below 170° C. It will be seen, furthermore, that when the percentage of oils in the distillate falls below 35 or 30 per cent these oils consist altogether of the heavier ones which distill above 170° C. Therefore, the conclusion drawn from Table 7 is that when the distillate contains 55 per cent or more of oils, these will comply with the requirements for a high-grade uniform wood turpentine, 90 per cent of which will distill below 170° C. When from 55 per cent down to 30 per cent of the distillate consists of oils, considerable quantities of light oils are present and the oils must be redistilled with steam, probably only once from a column still, to separate the light oils, which should, as before, constitute 55 per cent of the distillate. That portion of the distillate containing less than 30 per cent of oils contains only heavy oils, or pine oils, and need not be again distilled to separate oils boiling below 170° C. To this portion may be added the oils constituting less than 55 per cent of the distillate, of the redistilled middle fraction, or, if desired, this portion may be kept separate, as the oils are somewhat lighter than the heavy oils from the first distillation.

Table 8 contains the data concerning the several fractions obtained in the different distillations of the crude oils. These data are of the same general nature as those in Table 7. The temperature of distillation, proportion of turpentine in the distillate, specific gravity, refractive index, and behavior on redistillation are in general harmony, as is to be expected. In general, the larger the percentage of the crude turpentine contained in the first or A fraction and the smaller the percentage of turpentine in the distillate, the higher the specific gravity and the smaller the quantity passing over below 170° C. on redistillation. The figures also indicate that those fractions of which fully 90 per cent passed over below 170° C. on redistillation constituted at least 54 per cent of the distillate from the refining still. Those fractions which constituted from 54 to 30 per cent of the distillate contained large quantities of heavy oils, and a second distillation was necessary to separate them and obtain a product 90 per cent of which passes over below 170° C.

The data under barrels 7 to 11A, 7 to 11 A+B, and 1 to 6 A (Tables 7 and 8) which were mixtures of first or A fractions, show that on a second distillation an entirely acceptable wood turpentine was obtained, and that approximately 92 per cent of the first fraction was light oil. Approximately 50 to 54 per cent of the crude oil was recovered in the first distillation as a high-grade wood turpentine. The highest figures, those on barrel 3, indicated that the crude turpentine employed did not contain more than 60 per cent of oils distilling below 170° C, while those on barrel 4, which are considered the most reliable, having been corrected for variations in pressure and temperature, indicate 54 per cent of light oil distilling below this temperature. This figure is probably correct within 2 per cent. That is, the crude oil contained from 52 to 56 per cent of light oils distilling below 170° C. The separation in a single distillation of a uniform commercial fraction containing 90 per cent of these $\left(\frac{90\times54}{100}\right)$ or 48.6 per cent instead of 54 per cent, may be considered a satisfactory separation, as it leaves in the heavy or pine oils 5 per cent of the original light oils. This is, as will be seen by consulting the tables, concentrated in a middle fraction constituting from 15 to 30 per cent of the original crude oil. Of this middle fraction 16 to 33 per cent is light oil, which can be almost completely separated by a second distillation with steam in a column still. This second separation will probably be sharper than the first, for the reason that by far the greater part of the heavy oils present in the crude oil has been removed, and of course the distillation is no longer affected by them.

USE OF THE POT STILL.

Similar, but less complete, experiments were conducted in Georgia using an ordinary gooseneck turpentine still for refining crude steam-distilled wood turpentine. These stills were charged with between 350 and 400 gallons of crude oils and refined with a slow current of live steam, from 10 to 12 hours being required to distill off the wood turpentine from the pine oils. The distillate, consisting of water and turpentine, flowed from the condenser at a quite uniform rate of 1 gallon in from one minute to one and one-fourth minutes. As it was not feasible to measure the distillate, the refined wood turpentine, nor the pine oil recovered from the charge, these can only be calculated from the rate of distillation for the three experimental runs. These results are only approximate, but show roughly the quantity of heavy oils passing into the wood-turpentine storage tank.

The results obtained with the ordinary pot still having a gooseneck (Tables 9 and 10) agree quite well with those from the column still in indicating that the percentage of turpentine in the distillate must not fall below 54 if 90 per cent of the refined product is to distill below 170° C. Also that the portion of the distillate which contains between 54 and 30 per cent of oils is a mixture containing much pine or heavy oils, which should be again distilled to separate the turpentine from it; and, finally, that when the distillate contains less than 30 per cent of oils, these oils are not turpentine but pine oils.

In these experiments, the distillation of the so-called turpentine may be roughly divided into three periods; the first covering somewhat less than a third of the time, during which the light oils (90 per cent of which distill below 170° C.) are passing over; the second period covering from one-third to two-fifths of the total time, when a mixture containing larger and increasing quantities of heavy oils is distilling; and the last period, approximating one-fourth of the time, when heavy oils which should properly go to the pine-oil tanks are distilling.

Calculations based on the refining data indicate that the crude oils contained approximately 70 per cent of wood turpentine distilling below 170° C. Calculations from the factory records and the analysis of the refined wood turpentine showed 60 per cent of wood turpentine in the crude oils. The apparent discrepancy is doubtless due to the fact that no accurate measurements of volumes are available for calculation.

A comparison of the data in Tables 6 and 10 shows that the samples taken from the column still and from the pot still, when the percentage of turpentine in the distillate was the same, are much alike in composition. It must be remembered in comparing the data that the percentage of turpentine in the distillate can not be determined with great accuracy. The figures given may be in error 2 per cent. Further, the distillation of the samples from the column still were made with emergent thermometers and were not corrected for pressure.

On the whole, the data from the two forms of stills are very concordant. They agree in indicating that as long as the distillate from the refining still consists of at least 54 per cent oils, 90 per cent of those oils will distill below 170° C.; that when the distillate contains from 54 to 30 per cent of oils, the oil is a mixture of light and heavy oils, which must be again distilled to separate the light oils distilling below 170°. When the distillate contains less than 30 per cent of oils, it contains practically no oil distilling below 170°. The specific gravity of the oils bears a fairly close relation to the percentage of oils in the steam distillate. When the percentage of oils in the distillate is 55 to 53, the specific gravity is 0.8634 to 0.8638; when the percentage of oils is 30 to 27 the specific gravity is 0.8811 to 0.8868 (omitting one doubtful result).

The data can not be employed to show definitely the comparative efficiency of the column and pot stills in refining wood turpentine. They do indicate, however, in a general way that the fractionation is sharper and that the intermediate fraction of the light and heavy oils is smaller from the column than from the pot still. It will be noted that in the earlier stages of the distillation from the column still, the distillate contained from 54 to 65 per cent of oils, while the distillate from the pot still contained only 54 per cent. This indicates, as has been said, a much sharper separation on the part of the column still during the first of the distillation. If the distillation were stopped at any given point, say when the percentage of turpentine in the distillate was 50, the separation in the column still would be more complete and the turpentine from it would contain less heavy oils than that from the pot still. It has been stated that from the column still a middle fraction containing about 5 per cent of the light oils that were in the crude oils is obtained. Calculations based on the rate of distilling indicate that the middle fraction from the pot still contains from 10 to 16 per cent of the light oils present in the crude oils, and that it is larger than the middle fraction from the column stills.

Experiments similar to the foregoing were also conducted with destructively distilled turpentine, and results of the same general nature as to the separation from the heavy oils, percentage of oils in distillate, etc., were obtained. The stills used in this work became unavailable at this time and therefore no effort was made to separate the light oils, distilling below 150° C., other than to make three fractions of the distillate. The first fraction should have contained the lighter oils with but small proportions of turpentine, the second fraction the wood turpentine, and the third the pine oils, or, more properly, the heavier constituents of rosin spirits. The separation between the light oils and the turpentine was not at all sharp, and a single fractionation in the column still did not give in any case a fraction which contained all the light oils, with but minor percentages of turpentine.

If distillation of the first fraction was continued sufficiently long to carry over all the light oils, it contained 50 per cent or more of turpentine, or if the distillation of the first fraction was discontinued when it had but a small proportion of turpentine, it did not contain all the light oils.

Between the turpentine proper and the heavy oils there was also a mixed portion, as in the case of the steam-distilled turpentine, and this contained notable percentages of light constituents. It is quite evident, therefore, that, when operating these stills in the manner described, it is necessary to make a second distillation of two middle fractions, one to separate rosin spirits from turpentine and one to separate turpentine from the heavy oils. Under the circumstances, it is not deemed advisable to include the detailed figures on the distillation of the destructive turpentine.

CONCLUSIONS IN REGARD TO REFINING.

From the data obtained in these experiments it is concluded that the percentage of oils in the distillate furnishes the most reliable and useful information as to the progress of distillation and the nature of the oils at different times, and this is also the simplest means of acquiring such information. Neither the specific gravity of the oils nor the temperature of the distilling vapors at the top of the still furnishes as reliable information, nor are they so conveniently determined. The temperature of the vapors is materially affected by the volume and temperature of the steam entering the still and by variations in atmospheric pressure. The former can of course be made practically constant by the use of reducing valves, but corrections for atmospheric pressure require more training, experience, and care than can probably be given at such plants.

The specific gravity of the samples taken during distillation often shows a decided conflict with the other data on the samples, but in the main it is a reliable indication of the progress of the distillation and the composition of the oil. But as this determination also requires more skill, and is in every way more difficult to make in the works, it is not as safe a guide as the volume relations of the distillate.

The column still will give sharper separations of wood turpentine from pine oils than can be obtained with the pot still, but the experiments here described do not indicate any great superiority of the column. The data available, however, are inadequate to permit the drawing of definite conclusions as to the economy or efficiency of the two forms of stills.

VARNISH AND PAINT EXPERIMENTS.

ANALYSES OF THE TURPENTINES AND VARNISHES EMPLOYED.

In order to have a strictly comparable series of experiments on the value of wood turpentine as a thinner, varnishes and paints were prepared and thinned with several different turpentines. That there might be no question as to the authenticity of the samples, the author personally collected them and was present when the paints and varnishes were made. Four turpentines, such as are usually found on the market, were used in these experiments—a gum spirits, a steam-distilled turpentine, and two samples of destructively distilled turpentine, both of which had been distilled after washing with soda. None of the wood turpentine was as good as can be prepared by careful refining; all except No. 3 contained much heavy oil which gave them a marked odor and undoubtedly made the varnishes dry more slowly. Results with these turpentines, therefore, would naturally prove more unfavorable to wood turpentine than if the properly refined article had been used. Analyses of the turpentines used in these experiments are given in the following table:

Analyses of the turpentine used in varnish experiments.

Determinations.	Gum spirits.	Steam distilled and refined.	Destructively distilled, washed with soda, and steam distilled.	
	No. 1.	No. 2.	No. 3.	No. 4.
Specific gravity at 20° C.	0.866	0.871	0.860	0.857
Angular rotation at 20° C.	+4.20	+33.4	+11.5	+16.4
Index of refraction at 20° C.	1.4698	....	....	1.4723
Initial distilling temperature °C.	155	156	154	162
Distillation tests:				
Distilling below 160° C. (per cent)	82	26	63	0
Distilling between 160° and 170° C. (per cent)	13	48	29	65
Distilling between 170° and 185° C. (per cent)	2	12	2	30
Residue above 185° C. (per cent)	2	13	5	4
Total iodin absorption	431	362	398	352
Iodin absorption, addition	335	294	322	282
Iodin absorption, substitution	48	34	38	36
Acid number	0.347	0.270	0.110	0.040
Saponification number	3.41	7.72	3.92	0.9
Color in 200 mm columns:				
Yellow	0.74	5.0	1.60	0.84
Red	0.2	....	....	....
Polymerization residue (per cent)	<1	<1	<1	<1

It will be noticed from these analyses that according to the distillation tests the gum spirits and one of the destructively distilled turpentines should be ranked as quick-drying turpentines, with the advantage in favor of the gum spirits; while the steam-distilled and the other destructively distilled turpentine are slow-drying, the latter being the slower in the initial stages, but the former requiring longer to dry hard. It should be noted that 74 per cent of the steam-distilled turpentine distills below 170° C., i. e., 26 per cent of the turpentine is nonvolatile at that temperature; yet, as will be seen subsequently, the varnishing tests do not indicate that the varnish thinned with this turpentine took longer to dry than the others. Some observers reported that this varnish dried more slowly than the other samples, while others stated that it dried fully as fast. The comparatively low acid and saponification numbers of the turpentine refined from soda are to be expected. The steam-distilled sample is of deeper color than the others, but no difference can be detected in the color of the varnishes, which can be ascribed to the color of the turpentine used. While the analyses given indicate that there is a marked difference in the behavior on distillation between the several turpentines, this difference is no greater than that frequently observed between samples of gum spirits.

Two classes of varnishes were prepared and thinned with these turpentines; a coach finishing varnish to represent outdoor conditions, and a piano varnish to represent those used in interior work. These were prepared by two experienced makers under the writer's personal supervision, in accordance with their regular formulas for such varnishes. In preparing them the gums and oils were cooked in the usual way, and when sufficiently cooled portions of the batches were thinned to the desired consistency with each of the described turpentines. For comparison, in order that there might be no possible differences due to a variation in manufacture, a portion was also thinned with the turpentine regularly used by the firm. These varnishes were allowed to age for one year at an even temperature, when they were racked off and half-pint samples sent to prominent varnish makers, piano makers, and carriage builders, who had consented to make panel tests of the working qualities and to pass judgment on the merits of the different varnishes.[1] Small quantities of the turpentines were sent with the different varnishes that they might be thinned before using, if necessary, with the same turpentine used in its preparation. The firms which made these tests also prepared a panel with the varnish they regularly used for each class of work. There were thus standard varnishes thinned with gum spirits, two of which were identical, except as to the turpentine, with the three thinned with wood turpentine, while the sixth varnish was that with which the various varnishing workmen were familiar and with which they were securing satisfactory results. Very complete data were requested as to the working, behavior under the brush, tendency to check or peel, time of drying, difference in gloss, behavior when rubbed, disagreeableness to workmen, and other pertinent facts, all of which will be found in Table 11, page 69.

Extended analyses of the varnishes used in these tests has not been attempted because they would possess but little significance. It is in the preparation of the varnishes that the difference in odor between gum turpentine and wood turpentine is most strongly brought out, as it is much more pronounced at this stage than in the cold turpentine or in the completed varnish at ordinary temperatures, though here, too, it is quite easy to differentiate the several varnishes. The odor of the hot varnish thinned with the steam-distilled turpentine was much more objectionable to the writer than that of the varnishes thinned with the destructively distilled samples. In the former case the odor can be best described as resinous and nauseating, while in the latter case it was resinous, penetrating, and smoky.

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[1] It was assumed that the finished varnishes made from the same batch of oil and gum would have practically the same consistency; this was not the case.

The viscosity of the varnishes was determined by the Doolittle viscosimeter and found to be as follows, when expressed in angular degrees of retardation:

Viscosity of three kinds of varnish (expressed in angular degrees).

Varnish No.	Coach finishing.	Piano rubbing.	Piano flowing.
1	135.7	117.0	69.4
2	69.1	121.7	64.7
3	72.9	97.5	62.0
4	62.1 61.5	78.7	50.2
5	91.4 92.2	96.2	80.5

That is, the different samples of the same kind of varnish were not at all alike in consistency. This fact has but little significance, however, except that it made it necessary for each workman to thin the varnishes himself before using them. It is more than probable that this would have been done in any case and the final results do not indicate that the differences in consistency had any practical effect on the finished coat. It will be seen from the following table, and from Table 11, page 69, that the experimenters seldom thinned the varnishes in conformity with their determined viscosity. Neither is there any apparent connection between the turpentine content, the thinning required, and the time of drying of the different varnishes.

Order in which the varnishes should be thinned.

Coach finishing.	Piano flowing.	Piano rubbing.
No. 1	No. 5	No. 2
No. 5	No. 1	No. 1
No. 3	No. 2	No. 3
No. 2	No. 3	No. 5
No. 4	No. 4	No. 4

The percentage of volatile matter distilled from the varnishes is shown in the following table:

Percentage of volatile matter distilled from the various varnishes.

Various thinners used.	Coach varnish.	Piano rubbing varnish.	Piano flowing varnish.
	Per cent.	*Per cent.*	*Per cent.*
Gum spirits	59.4	64.3	53.8
Steam-distilled wood turpentine	45.8	64.0	54.7
Destructively distilled, soda-refined turpentine, 3	51.1	65.0	57.2
Destructively distilled, soda-refined turpentine, 4	47.7	63.4	55.8
Gum turpentine	51.6	66.9	53.9

Perhaps the only point worthy of attention here is the large amount of volatile matter contained in the first coach varnish thinned with gum spirits. This is the varnish that has the highest viscosity and required the greatest amount of thinning.

The color of all the varnishes was determined, using the Lovibond colorimeter. Readings were made in the ¼-inch cell. All varnishes were found to have the same amount of yellow, the only variation being in the red readings.

Color determinations on varnishes thinned in different ways (Lovibond readings).

Thinners used.	Coach varnish.		Piano rubbing.		Piano finishing.	
	Red.	Yellow.	Red.	Yellow.	Red.	Yellow.
Gum spirits	11.45	15	9.80	15	11.90	15
Steam distilled and refined	9.80	15	9.80	15	11.90	15
Destructively distilled and soda refined, 3	9.60	15	10.60	15	12.00	15
Destructively distilled and soda refined, 4	10.05	15	9.75	15	11.40	15
Gum spirits	10.15	15	9.90	15	10.60	15

No useful conclusion can be drawn from the color tests. The data furnished by the various experimenters are brought together in Table 11.

COMMENTS OF THOSE WHO TESTED THE VARNISHES.

Murphy Varnish Co.: We have reached the conclusion that the coach and piano finishing varnishes have been mixed. If it is so, our tests, so far as drying and rubbing properties are concerned, are valueless. Generally speaking, we are of the opinion that the samples you sent us are poor varnishes; poor as to materials and poor as to methods of manufacture.

A. B. Chase Co.: In our opinion these varnishes (piano) were prepared and thinned with a turpentine substitute. When these varnishes were polished they did not come up to our expectation, so we concluded they were gloss varnishes and simply gave the panels another coat and left them with a gloss finish.

The James & Meyer Buggy Co.: As far as we can judge without giving them one year's test in the open weather, No. 1 and No. 3 turpentines could be used by us about the same as regular turpentine. No. 2 and No. 4 could not be used at all, as they crawl apart under the brush.

Steinway & Sons: None of the varnishes is as good as that regularly employed by this firm.

Vose & Sons Piano Co.: We attempted to make the panel tests, following out your instructions, and are sorry to report that for all purposes the samples sent us were entirely impracticable. Some of the panels, in fact nearly all of them, still remain tacky after the considerable length of time since it was applied. It is entirely impossible to rub them.

DISCUSSION AND SUMMARY OF THE VARNISHING EXPERIMENTS.

COACH-FINISHING VARNISHES.

All experimenters thinned one of the varnishes made with gum spirits, three thinned all varnishes the same, and one thinned both gum-spirits varnishes. The other varnishes were not thinned.

The time that the varnishes remained tacky varied from 3 to 36 hours, and the results do not show that the wood turpentines behave materially differently from the gum spirits in this respect. The varnish thinned with the wood turpentine containing the most heavy oil (No. 4) does not remain tacky materially longer than the others.

The same statements apply to the time which is required for the varnish to dry hard; some found that all dried in 8 to 12 hours, others reported 12 hours, 24 hours, and 54 hours. Three experimenters reported the same drying for all the varnishes.

Three reported a difference in gloss; one, that the varnish thinned with the destructively distilled turpentine (No. 4) had the best gloss; another, that the gum was superior to the wood turpentine, and the third, that all the wood-turpentine-thinned and one gum-thinned varnish showed tears.

All observers detected the wood turpentine by the odor, and found the varnishes thinned with them objectionable because of this.

Only one observer raised any other objection to the varnishes; this one found the wood turpentine varnishes light in body.

Only one observer found any difficulty in working the varnishes; in this case the gum turpentine and the destructively distilled wood turpentine (No. 4) having the least residue were found objectionable.

In rubbing, one observer found all unsatisfactory; two found all satisfactory, and the others did not report specifically.

As to behavior under the brush, the results do not lead to any conclusion, as different observers express contradictory opinions as to the behavior of the same varnish. This is a working condition, however, which probably has but little to do with the nature of the varnish. Two observers find one of the gum-turpentine varnishes rather heavy in body; one finds the wood-turpentine varnishes thin and light in body; one finds that the varnish thinned with the destructively distilled turpentine (No. 3) loses luster, that all of them are silky in appearance, and that the one thinned with the destructively distilled turpentine (No. 4) leaving the least residue is the best Another observer finds in striping that one destructively distilled turpentine (No. 3) works like regular turpentine, while the steam-distilled (No. 1) and the destructively distilled turpentine leaving the largest residue (No. 4) crack and crawl apart. This observation does not appear to have been made by the other experimenters and would seem to be connected in some way with the pigment present in the material used by this experimenter. It will be noted that these are the samples that contain large percentages of heavy oils not distilling below 170° C.

PIANO-RUBBING VARNISHES.

One experimenter did not thin the varnishes, one thinned one gum-turpentine varnish only, three thinned all varnishes alike, and two used different quantities of turpentine in thinning the varnishes.

In some instances the varnishes thinned with the wood turpentine remained tacky longer than the varnishes thinned with gum spirits; in others the reverse was true. In no case did the thinning agree with the viscosity tests of the varnishes. The time of drying varied but seemed to bear no relation to the kind of turpentine used in the varnish or to the viscosity of the original varnishes. In some instances the wood turpentines were much slower than the gum turpentines, while in others there was reported no practical difference in this respect.

With regard to luster, three found some difference among the varnishes. One reported that the steam-distilled wood turpentine (No. 2) had the best luster, the destructively distilled (No. 3) being second best. Another found one gum and one wood turpentine-thinned varnish had medium gloss, and one gum and one wood turpentine-thinned varnish had fair gloss. The other observers distinguished no difference in this particular. One found that the steam-distilled turpentine (No. 2) gave the best gloss, while one gum (No. 1) and one destructively distilled product (No. 3) showed tears.

Two found the odor of all the wood turpentine objectionable; one found the steam-distilled (No. 2) and one destructively distilled (No. 4) objectionable. One found both destructively distilled samples objectionable, while another said that the No. 1 gum-turpentine varnish had the odor of naphtha.

No difference was detected in working qualities, except that one observer found that the varnishes thinned with the destructively distilled turpentine (No. 3) worked unsatisfactorily.

All rubbed down well, one observer reporting in favor of the gum spirits. Three observers found no difference in behavior under the brush, while two others found some difference. One reports sweating.

As to body, there appears to be no difference that can be correlated with the turpentine used. One firm expresses the opinion that all the varnishes rub better than the varnish they regularly use; another, that only time and exposure make it possible to determine accurately the value of the varnishes; another, that they find it very difficult to form an opinion on the merits of the varnishes. The wood turpentines are said to contain very strong elements of resinous spirits, from which the best results can not be obtained.

PIANO-FINISHING VARNISHES.

One experimenter thinned both gum turpentine varnishes and that made with steam-distilled turpentine (No. 2); another thinned only one gum spirits sample. Two observers did not thin the varnishes, and two thinned all alike. In no case was the thinning done in the order of the viscosity of the several varnishes.

The varnishes remained tacky for from 4 to 55 hours, but there was no relation between this condition and the viscosity of the varnishes or the distillation tests of the turpentines.

The data do not indicate that there is any difference in the time required to dry hard. No difference in gloss was reported, except in one instance a gum turpentine varnish is stated to have the poorest gloss. One observer finds tears in Nos. 1 and 3.

In one case the odor of the destructively distilled, soda-refined sample was reported as objectionable; another objected to the odor of both destructively distilled samples Nos. 3 and 4, and a third observer detected all the wood turpentines, and stated that both of the gum spirits varnishes had an old fatty oil odor. The odor of the steam distilled and one gum turpentine was found objectionable in one case and in another both destructively distilled samples were so reported.

Only one observer found any difficulty in working the samples, the destructively distilled wood turpentines being difficult to handle.

None of the varnishes was found satisfactory in polishing, except by two observers.

Two observers found that all of the varnishes flowed freely from the brush; one that there was some difference. One observer found all the varnishes unsatisfactory, as they did not flow out well. The one thinned with gum spirits (No. 1) was best, the varnish thinned with destructively distilled turpentine (No. 3) was rated second, while the steam distilled (No. 2), the other destructively distilled (No. 4), and the other gum spirits (No. 5) varnishes were found to be poor. No choice could be made between the samples. The same observer remarks that in his opinion all these varnishes were prepared and thinned with turpentine substitutes. Another observer found all the varnishes unsatisfactory as polishing varnishes, the two destructively distilled samples being the worst. Another observer finds the samples from fair to good in all respects, with those thinned with gum spirits the best in every respect.

All but one of the experimenters recognized that No. 1 coach varnish was the most viscous, and thinned it more than they did the other coach varnishes. One also thinned No. 5, which was the next most viscous varnish. None of the experimenters was able to detect working differences in the consistencies of the other samples of coach varnish. Three experimenters apparently attempted to thin the piano rubbing varnishes to approximately the same consistency; only one, however, approached the order indicated by the viscosity determination. Considering the results as a whole, it is not possible to state that any of these varnishes behaved materially differently from the others either in drying or under the brush. The observations of two experimenters are in harmony with the viscosity tests, but those of the others are not. Where a difference in luster was observed the wood turpentines were best.

None of the experimenters thinned the piano flowing varnishes in the order indicated by their viscosities. On these varnishes also the data do not warrant any conclusion as to the differences in their behavior on application, and no practical difference in gloss was observed. These, being flowing varnishes, were not intended to be rubbed, but all of the experimenters, through misunderstanding, rubbed the panels, usually with very unsatisfactory results.

In making a careful study of the mass of data obtained in this work one is at once impressed with the many different ways in which these varnishes were handled from the thinning to the finishing touches, and by the seemingly contradictory opinions which are drawn regarding them. There is no one point on which a decided majority agree except as to odor, and even here some do not object to the wood turpentines, while others complain of the gum spirits. So at variance are the reports that the conclusion is almost forced upon one that a workman's opinion of a varnish is largely influenced by whether or not it may be manipulated as he is used to handling varnishes under his working conditions. Other factors influencing the quality of varnish do not enter here because these samples are identical except as to the character and amount of thinner used. The above conclusion is not only supported by the contradictory reports, but also by the statements made by some that none of the varnishes was satisfactory.

It is rather surprising that no marked differences between the individual samples of the same class are definitely indicated in the reports of the several workmen. The determined viscosities of the several varnishes of a class indicate the order in which they should be thinned to bring them to the same consistency, or make them flow alike. The turpentines employed differed greatly, however, in their drying properties. The analyses indicate that Nos. 1 and 3 were quick-drying turpentines, while Nos. 2 and 4 were slow drying. In general, therefore, more of turpentines Nos. 2 and 4 would be required to thin a varnish to a desired consistency than of Nos. 1 and 3, but the resulting varnish should be slower in drying. It will be observed as a matter of fact that No. 4 varnish, in which destructively distilled turpentine was used, is the thinnest varnish of all and it was unnecessary to thin it further.

On the whole, no definite conclusion can be drawn from the data obtained by the experimenters on the handling and working of the varnishes. This is not a criticism of those who at considerable pains and inconvenience cooperated in the work. The unsatisfactory character of the data arose in part through the difficulty of explaining clearly in written directions exactly what experiments and what information were desired. Had it been feasible for a representative of the bureau to talk with and assist those actually doing the work, more valuable information could doubtless have been obtained. Of course the data could only be of value in determining the relative working qualities of the varnish thinned with the different turpentines; but little information as to the appearance and durability of the varnish was obtained. Opinions on this point must be based on a later careful examination and study of the finished panels. These panels were prepared four years ago. Since that time they have been exposed in one of the office rooms of the bureau for from 12 to 18 months, and during the rest of the time have been stored, protected from dirt and light. None has been exposed to the weather at any time.

Careful examination of these panels by a number of persons, including master painters and others familiar with the use of varnishes, discloses no marked deterioration of any of the coats nor any clear-cut difference between the appearance of the several varnishes of the same class, except that as a rule the varnish which the workman was in the habit of using regularly looked a little better than the others. This fact confirms the statement previously made that the familiarity of the workman with the varnish influences the success of his work, and, consequently, his opinion of the varnish.

In addition to these experiments the same turpentines were employed in thinning a white lead and linseed oil paint. No difference was observed in the working of the paint, and after drying all painted panels presented the same appearance. There was no difference in the color of the panels after one year. The interior woodwork of the new building of the Bureau of Chemistry was painted with zinc oxid and linseed oil thinned with other steam and destructively distilled turpentines. The workmen stated that the odor, particularly of the destructively distilled samples, was unpleasant, but it did not seriously inconvenience them or make them ill. Both turpentines (also a light pine oil) behaved well under the brush and no objectionable features in working other than the odor were observed. After 18 months there is no apparent difference in the paint thinned with the different turpentines.

About five barrels of refined wood turpentine, which had been obtained in the refining experiments, and of which approximately 75 per cent distilled below 170° C. were turned over to the painter of the department and used by him on all classes of paint and varnish work from ordinary house painting to high-class furniture varnishing. No inferiority in the finished work or in behavior in applying was observed by the master painter. Only one workman made complaint regarding this material, claiming that it made him ill. The statement was not investigated.

COMMERCIAL OPINIONS AS TO THE VALUE OF WOOD TURPENTINE.[1]

Before entering upon the research work on this subject the opinions of those having occasion to use turpentines in large quantities was asked as to the value of wood turpentine as compared with gum spirits.

OPINIONS OF WOOD TURPENTINE PRODUCERS.

Cheraw Naval Stores Co. (Inc.), Cheraw, S. C.: Our product is used largely by paint men in the South, * * * who claim that it is better paint spirits than the old-process turpentine.

F. L. Huggins, Wilmington, N. C.: Wood turpentine dries better than ordinary turpentine. * * * The only difference is in the odor.

Morris Weslosky, Albany, Ga.: Its solvent power is as great, it dries quicker, and varnish made from it is no more difficult to work than that made from ordinary turpentine, but is more liable to check. Painters object to the odor and state that the acid that seems to be left in it hurts their eyes. Consumers object only to the odor.

Weed Distilling & Manufacturing Co., New York: We have heard no objection to our products which are taken by the trade at the market rates. The oils for varnish work are more readily sold every day.

Chesterfield Naval Stores Co., Cheraw, S. C.: No objection has been raised to the material.

Tyler Lumber Co., New York: We find that the solvent property of wood turpentine by the steam process is greater than that of gum turpentine. It will not dry as quickly as gum, taking about two hours longer. It has been claimed that for carriage varnish, etc., the varnish is not so flexible as when gum spirits are used. The only complaint of any moment is made by painters and other consumers on account of the odor which is objectionable for interior work where the building is closed.

Pensacola Tar & Turpentine Co., Pensacola, Fla.: The solvent power of wood turpentine is the same as the old fashioned article. It dries as well, and there is no difference in its working qualities. The only objection is that the odor is unpleasant. Its power of absorbing oxygen seems to be equal to any turpentine.

Naval Stores Supply Co., Biscoe, N. C.: We have been selling practically our entire output to users of paints, and we suppose that this turpentine was used exactly as the turpentine made by the old process. The only objection that we have heard is with regard to the odor.

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[1] These statements are not in all cases quotations but are condensed from correspondence. The Bureau of Chemistry is not in any way responsible for these opinions.

Spirittine Chemical Co., Wilmington, N. C.: The solvent power is the same. It dries as well and there is no more liability for varnishes thinned with it to check than those made with gum spirits. There is a difference in odor, a slight difference in color, but neither enough to injure its usefulness. We have as customers for this product many of the leading paint and varnish manufacturers and they have been buying to their complete satisfaction for many years.

OPINIONS OF PAINT AND VARNISH MAKERS.

Murphy Varnish Co., Newark, N. J.: Our tests of this material have shown that it is not so well adapted to varnish-making purposes as pure spirits of turpentine. It is objectionable on account of the odor and also because less of it is required in the manufacture of gum varnishes than the pure spirits of turpentine. As turpentine is the cheapest ingredient it is obvious that the cost of such varnishes is increased by its use.

The Glidden Varnish Co., Cleveland, Ohio: We are experimenting with wood turpentine continually, but up to the present time have been unable to find any product that was entirely satisfactory in odor. The proper deodorization of wood turpentine would no doubt make this product as valuable as pure gum spirits. In all of our experiments with a high-grade wood turpentine we find that the working properties of same are equal to those of gum spirits, but wood turpentine gives varnish or paint a very pungent odor which is very disagreeable to the user.

Harrison Bros. & Co. (Inc.), Philadelphia, Pa.: Properly distilled wood turpentine free from pyroligneous impurities has equal or better solvent properties than gum spirits. It dries as well, but possibly a little slower. Varnish made with it is in no way inferior to one made with gum spirits so far as working and physical properties are concerned. The principal objections raised by painters and consumers are the odor and irritating action of the vapors upon the eyes, while some painters claim that it also produces nausea.

Chicago Wood Finishing Co., Chicago, Ill.: The solvent power of wood turpentine is equivalent in every way to gum spirits. In drying it is not so much slower than there would be any great disadvantage. Mixed with varnishes we find that it is equivalent to gum spirits when mixed in any quantity of almost any kind of varnish in which a gum spirit would be used. It does not differ, as far as we have seen, in working qualities from gum spirits. The principal objection is its extremely pungent odor which, to most people, is very disagreeable, and in some cases causes violent headaches. Because of this odor we prefer the gum spirits.

Longman & Martinez, New York City: The best quality of wood turpentine evaporates much slower than gum turpentine, and were it not for the odor of wood turpentine it would probably be as well suited for use, to as large an extent, in paints as is gum turpentine. Neither is of any value whatever in respect to furnishing durability to paint. The odor of even a good quality of wood turpentine is not so often an objectionable feature.

Atlantic Drier & Varnish Co., Philadelphia, Pa.: The solvent power is equal. It dries as well and does not differ in manipulation from ordinary turpentine. It does not make an inferior varnish, and paint or varnishes made with it do not differ perceptibly in covering power from ordinary turpentine. Painters, consumers, and workmen all agree in objecting seriously to the strong odor and to its bad effects on the eyes. It is very much more offensive in every way than ordinary turpentine. We have found such serious objections because of the odor that we have abandoned the use of the material entirely.

Boston Varnish Co., Boston, Mass.: We have not gone into the flexibility or life of wood turpentine, but in our opinion from what we have used it is equally as good as regular turpentine. In our opinion a varnish reduced with it would produce a product that would be absolutely impossible for any painter to use in any quantity in a close room. The principal objection to it for medium and high-grade varnishes is its odor. The above remarks apply only to the pure, properly made, and well-refined article.

C. A. Willey, New York City: From careful experiments in a practical way I am fully convinced that it will do everything that an ordinary turpentine will do, but the trouble is with its odor, of which the painters immediately complain so that we were obliged to discontinue its use on this account. Recently there is much improvement in the odor of some samples.

Charles H. Howell & Co., Philadelphia, Pa.: From some little experience we have come to the conclusion that barring the smell, the dark color of that which has simply been distilled, it is a good product, answering all purposes of gum turpentine, with only the objectionable odor against it. Our experience with the clear white article which we think is produced by treating with caustic is that it is utterly worthless, being very detrimental to paint, and especially to oil paint. Such treatment takes the greater part of the oil out and leaves an article no better than benzine. It contains a strong alkali which forms a soap of the oils in paints, and kills their durability.

Standard Varnish Works, New York, N. Y.: For our requirements we find this article to answer the same purposes as spirits of turpentine, the only noticeable difference being the odor, to which the trade seriously objects.

Berry Bros. (*Ltd.*), *Detroit, Mich.:* We do not and never have considered distilled turpentine as being equal in any respect to old process goods.

Emil Calman & Co., New York, N. Y.: We find that for many purposes it will replace the gum article. Its solvent power appears to be about the same and when properly manufactured it will dry as well as ordinary turpentine. We do not find any material difference in working qualities. There is objection from buyers of materials to the odor, which is stronger than that of gum turpentine. These remarks apply only to properly manufactured wood turpentine.

Boykin Manufacturing Co. (*Inc.*), *Cheraw, S. C.:* We have been marketing wood turpentine to the paint, furniture, and buggy manufacturers with success. We rarely have a kick on account of quality, and this class of trade claims that the wood turpentine does their work as well as the old process turpentine does.

The Institute of Industrial Research, Washington, D. C.: The solvent power of wood turpentine is high and is probably not less than that of gum spirits of the same specific gravity. When properly prepared and purified, it dries as well as gum spirits. From this point of view it is therefore a good varnish material and is said to work free and exhibit good covering power. The principal objections to wood turpentine are made on account of its unpleasant odor and lack of uniformity. It contains, if improperly purified, certain * * * products which are said to have a bad effect upon the kidneys of workmen who have to handle it. These properties when present also make it unfit for interior application.

John Lucas & Co., Philadelphia, Pa.: Perhaps definite or practical information on the subject of the commercial value of this product in comparison with the gum turpentine is not of a very reliable character. Experiments made or experience gained may not have been such as to fully determine the point. We have heard that varnish made from a wood product would, when applied over a varnish made from gum spirits, on account of its higher solvent nature, dissolve the surface of the previous coats and render the rubbing down almost impossible. In drying we have not noticed any particular difference. It has been our experience that a good grade of wood turpentine is equal in every respect to the gum spirits. A varnish properly made with wood turpentine of a satisfactory grade has just as good rubbing qualities as the gum-spirits varnish. The principal objection is its odor.

John W. Masury & Son, New York City: Aside from its objectionable odor there seems to be no reason why wood turpentine should not be used in paint as well as the gum turpentine. In the ripening or aging of varnish turpentine as well as linseed oil it absorbs some oxygen, * * * whereby the valuable properties of the varnish increase. * * * Here no solvent which does not have the capacity of such change can replace turpentine, but so far as we have been able to observe there is no great difference between wood and gum turpentine. We believe, therefore, that its pungent characteristic odor is the main obstacle to its universal substitution for the genuine in paints and varnishes.

As a problem apart by itself, however small may be the demonstrable scientific difference between wood and gum spirits, the conservative varnish maker will prefer the gum spirits. This very natural prejudice arises from his consciousness of unknown chemical relations in a process which for some time to come must remain largely empirical, and as his good reputation rests largely upon results so achieved he does not willingly take chances for the fraction of 1 per cent gain which the difference in price between the two turpentines has heretofore offered.

So far as paint is concerned the situation is clearer. Here turpentine is a volatile thinner, used to obtain a practical brushing consistency where it is desirable to keep the ratio of oil to pigment low.

Sherwin-Williams Co., Newark, N. J.: Our experience with the material is limited, but so far as we know the solvent power is not materially different. Careful tests have satisfied us that so-called wood turpentine does not dry as well as the gum spirits, but has a definite retarding action. In some instances this would not be objectionable; in others it would. So far as we know there is no other difference in varnish thinned with it nor in the manipulation or covering power of such paint and varnishes. We see no reason why the properly worked out process material should not be equal to ordinary turpentine.

F. W. Devoe & C. T. Raynolds Co., New York, N. Y.: The samples which we have examined have varied considerably, both in chemical and physical constitution. Some of them seemingly do contain a small proportion of the real turpentine, but most of them contain nothing other than what is probably known as pinene as distinguished from the terpenes of turpentine. For all practical purposes I believe that the solvent properties of the better grade would compare favorably with that of regular turpentine. Some of the better-made samples will evaporate with as little residue. The covering power of a paint or varnish made with wood turpentine should not differ in any way as far as I can see from that made with ordinary turpentine. The odor of the wood turpentine is so persistent that it can not be disguised. This odor is so disagreeable that we find painters and varnishers object to a varnish made with wood turpentine for the reason that it affects their eyes and also their throats, especially where the varnish is used in the interior of buildings where there is no draft to take away these odors.

Patton Paint Co., Milwaukee, Wis.: Considered as a paint thinner only, we believe a properly made wood turpentine is to all practical purposes just as good a thinner as gum turpentine. The principal objection that has been urged in the past against its use is that of its somewhat offensive and irritating odor. In the case of products of more recent refining processes these objections have been very largely if not wholly overcome; certainly reduced to such an extent that when present in the percentage found in ordinary paints no unpleasant results need be expected.

With regard to its utility as a varnish thinner we are not prepared to state as to whether in our opinion it would be safe to substitute wood products for gum turpentine on account of the turpentine being present in much greater quantities than in an ordinary paint. The objections previously urged are correspondingly more apparent. Moreover, any nonvolatile residue that might be contained in the wood turpentine would prejudice its use to a greater extent than in paint. When we refer to nonvolatile residue we consider the temperature at or about 70°.

National Paint, Oil and Varnish Association, New York City: The solvent power of wood turpentine is not less than that of gum spirits. If the gravity is the same it dries the same. It makes just as good varnish, it works just as free, its covering power is the same. The objections are purely a question of odor and uniformity. If it were made of uniform quality free from oil and poisonous acids it would be simply a question of educating the public to a different odor.

The Booth & Law Varnish Co., New Haven, Conn.: Wood turpentine has a decided value as a paint and varnish thinner and works as well as the gum turpentine in most cases. The principal objection to it in paint is the odor. To-day it is more uniform than formerly, almost equal to the best gum turpentine, and will answer as well in results.

OPINIONS OF USERS OF PAINTS AND VARNISHES.

Arnold Print Works, North Adams, Mass.: We have used wood turpentine in ordinary painting and find it gives entire satisfaction. It is much better for this work than many of the adulterated turpentines.

Magnus, Mabee & Reynard (Inc.), New York City: We find that wood turpentine in a number of instances will dissolve varnish gums at a lower temperature, and dries fully as well as the regular gum spirits of commerce. We have sold this product in carload lots to several large varnish manufacturers, who report to us that the results in using same are entirely satisfactory for the same uses that they have heretofore employed gum spirits of turpentine. When we first handled this product we occasionally had a few complaints because of its strong odor, but of late we have had no complaints at all, as our manufacturers are producing spirits of turpentine without the objectionable odor heretofore noted. We might add that there are various grades of wood turpentine, as the process of distilling same varies among different manufacturers. For instance, some use direct heat in the retort process, some use steam distillation, while others use a rosin bath. The last-mentioned process we consider produces the best product. Our customers are principally among the paint dealers and manufacturers of varnishes.

John A. Casey, New York City: The steam turpentine has a nice, sweet wood odor, and finds a more ready sale than the wood-destructive turpentine. It is our opinion, however, that as a solvent and drier the wood-destructive turpentine is to be preferred. We are still of the opinion that the wood turpentine will never really take the place of turpentine manufactured from the gum.

A good many varnish manufacturers are using, however, wood turpentine in connection with gum turpentine in large quantities, particularly within the last two or three years, as the price of turpentine manufactured from the gum is higher at the present moment than it has been any time since the Civil War. * * *

Of course we refer only to first-class goods and not to inferior goods. It seems that every wood-destructive plant makes a different article. When one process and only one will be employed and a uniform article can be put on this market, it will become a marketable article and of staple value.

Hartsville Furniture Co., Hartsville, S. C.: Have used a wood turpentine made by the destructive process, but abandoned it at once, as it made the eyes smart and burn. Odor not so objectionable.

W. W. Hines & Co., Petersburg, Va.: Can not sell on account of odor, particularly at present prices of gum turpentine (40 to 50 cents per gallon).

Hartsville Supply Co., Hartsville, S. C.: Do not have any difficulty in selling a destructive turpentine for painting purposes at 80 cents a gallon when gum spirits are 90 cents and $1.

Tanner Paint & Oil Co., Richmond, Va.: We have tried to introduce same in a number of instances, but the average workman will not work same. In closely confined places, such as boat bottoms, inside of locomotive tanks such as used on tenders, it is impossible for painters to use the wood turpentine on this class of work, as the odor from same is very nauseating in a closed place where there is no circulation of air.

Burckhardt Co., Cincinnati, Ohio: Our experience with wood turpentine has been very unsatisfactory. We find it almost unsalable on account of the penetrating odor. When a large surface is exposed freshly painted in a closed space, for example, any interior work, the fumes given off are exceedingly disagreeable, both as to odor and effect on the eyes. The natural gum turpentine is free from such objection.

A. B. Chase & Co., Norwalk, Ohio: Our experience with wood turpentine has been very limited. The only thing we noticed is that it curdled or precipitated the varnish, and that the odor was very offensive. We have decided that it is not to be used in thinning varnishes for use on high-grade pianos.

H. H. Franklin Manufacturing Co., Syracuse, N. Y.: We are of the opinion that wood turpentine would be of no use to us on account of the odor.

The Locomobile Co. of America, Bridgeport, Conn.: We have never been able to get satisfactory results in the paint department when using wood turpentine. Our painters state that it is very quick drying, has a tendency to thicken the paint after standing a short time, and on evaporation leaves the paint lifeless.

Brewster & Co., New York: We have only experimented with wood turpentine, but so far as we have gone we find that its solvent power seems to be less than ordinary turpentine, that it does not dry as well, and appears to leave a greasy residue on the surface of the article painted instead of drying flat and clear, as in the case where pure gum turpentine is used. It is also heavier to work. In covering power there is no appreciable difference. We have not found it satisfactory to use in our business where a very good finish is necessary.

Grand Rapids Chair Co., Grand Rapids, Mich.: Its solvent power is the same. It does not dry as well and makes a more inflexible varnish than ordinary turpentine. It is more difficult to work and appears to separate from the varnish. It deteriorates with age.

Newport News Shipbuilding & Dry Dock Co., Newport News, Va.: We beg to say that we are still using wood turpentine, or Florida spirits, and the only objection we find is that the odor is unpleasant, as before stated. If this bad odor could be eliminated, we would use it much more extensively.

Norfolk & Western Railway Co., Roanoke, Va.: We have found the wood turpentine to have as good solvent value as spirit turpentine, of slightly slower drying qualities, but would dry out in time giving good results. This time of drying has a practical value in many of our shop operations, and is ofttimes the determining factor. We would not consider it as good as spirit turpentine in varnish making, due to the presence in small quantities of creosote. We did not find wood turpentine difficult to manipulate, nor did we find it to give detrimental results so far as the covering value of the paint was concerned.

The odor is rather objectionable to painters, which is probably the only point so far determined on which we would not recommend its use.

Pullman Co., Pullman, Ill.: So far as we are able to see, the solvent power of wood turpentine is about the same as that of the ordinary turpentine, with the exception that it dries a little slower. The covering capacity is apparently the same as the ordinary turpentine, and we are unable to note any difference in its manipulation or working. No objection has been raised by any of our painters, and for all purposes it seems to be a good substitute for the gum turpentine.

Another prominent railroad company: During the past four or five months we have used samples of these products and there is considerable objection to them, principally on account of the odor, which is sickening. As to their solvent power we do not find there is any difference, nor do we find a difference in drying qualities. There is no difference in the work nor in manipulation. We notice in mixing these substitutes that they turn whitish as a foam which disappears when they are left to settle. From our short experience we should say that they would crack or check in time. In the case where turpentine paints are used—that is, coach colors where varnish is applied afterwards—we notice that they change the color. We do not advocate the use of wood turpentine.

In the foregoing opinions there is perfect agreement as to the objectionable odor of wood turpentine, although it would appear from some of the statements that some samples differ but little from gum turpentine in this property, a fact which is confirmed by the experience and observation of this bureau. As a rule the manufacturers of paints and varnishes state that properly prepared wood turpentine is fully as satisfactory as ordinary turpentine, drying as well and working as well as gum spirits.

Dealers are generally of the opinion that it is of practically the same value as gum spirits as a diluent, but find that the odor seriously interferes with the sale of the material.

Manufacturers state that they have no difficulty in disposing of their entire product to paint and varnish makers, and at a price close to that of gum spirits.

There is considerable difference of opinion among the users of paints and varnishes, piano makers, carriage and motor builders, furniture makers, railroad companies, shipbuilders, and house painters. Thus a prominent builder of automobiles states that their painters know very little about it, but that it is very quick drying and evaporates so rapidly from the paint that it is left lifeless. They have never been able to get satisfactory results with it. Another company states that it could not be used indoors on account of the odor. A piano company states that it curdles or precipitates the varnish and has an offensive odor. A shipbuilding company finds that it works as well and appears to give the same results as ordinary turpentine, and the only objection is its unpleasant odor. A railroad company finds it satisfactory as a paint diluent, but objects to its odor.

It would appear that the material differences in the expressed opinions as to the drying properties and suitability in general as a paint and varnish thinner are due in large part at least to the well known lack of uniformity in wood turpentines as heretofore placed on the market. It is more than probable that in those cases where decided objection is raised the turpentine contained considerable percentage of pine oils, which not only affect drying properties, but greatly increase the objectionable odor.

CONCLUSIONS.

POSSIBLE PRODUCTION FROM WASTE MATERIAL.

The waste wood of the South and Northwest from the lumber industry—tops, stumps, slabs, and sawdust, and the dead and down timber from fires and storms—furnishes one of the great undeveloped resources of this country. From this wood, by industrially developed chemical methods, the entire output of naval stores, embracing turpentine, rosin, tars, pitch, rosin spirits, and rosin oils, having an annual value of at least $30,000,000, may be obtained without boxing or turpentining a single live tree.

There is more than sufficient waste material to yield annually all the papers (except news), paper and box board, and building board required, for which wood is suitable.

More methyl alcohol, acetate of lime, and acetone can be produced from this wood than is now made in the country, and large quantities of ethyl alcohol may also be recovered. While it has not been proved that these four products can be made profitably from waste resinous wood, it is not at all improbable that the manufacture of one or more of them, in conjunction with other products, would be practicable.

There are millions of acres of cut-over land covered with stumps and dead and down timber, all of which, because of its resinous nature, decays very slowly, enduring for years. There is enough of such material to supply all demands for the above-mentioned products for a very long period. The processes, equipment, and technique for the utilization of this material are either in operation or may be readily devised.

LOCATION, MANAGEMENT, AND OPERATION OF PLANT.

The financial success of the production of wood turpentine and other materials from waste wood is not controlled by the yield of valuable products alone, but is also largely affected by the location of the works with reference to transportation of raw material, and the business ability and technical skill with which they are conducted. As labor and transportation charges are the chief elements in the cost of conducting these plants, they should be located as close to the supply of raw material as possible, in order that the cost of collecting and hauling the wood and disposing of the products may be reduced to a minimum. It is well to give the most careful attention to location; the plant should always command two independent avenues of transportation for raw material and finished products. Other things being equal, the cost of wood is least when the works are located close to a sawmill, as in this case much of the wood has already been collected for mill purposes, the wastes from which are available at the smallest possible cost. Mill waste, however, is seldom as rich in turpentine as what is known as "lightwood," so that if the mill is located at some distance from the lightwood supply it may prove more profitable to locate the plant at the lightwood supply and haul the mill waste. Each case must be decided after careful consideration of all the circumstances, and therefore more definite general advice can not be given than to advise locating as close to the raw material as other factors of cost will permit. Generally speaking, the operation of wood utilization works will prove more profitable when conducted under the same ownership or in close cooperation with the lumber industry, because of the cheap raw material available under these conditions, and also of the possibility of using the equipment, waste steam, power, etc., of the sawmill in assembling the materials and in the works. Large quantities of water are required in these plants for producing steam, operating condensers, etc., hence such plants should be located where a sufficient supply can be obtained. For these reasons wood turpentine should be one of the by-products of the lumber industry—furnishing another means of minimizing the dangers from the wastes of that industry and turning them to profitable account. Turpentine should not be the only by-product, however. The greatest returns will be obtained when several of the following substances are made from the waste woods of the yellow-pine lumber industry, namely, paper, wood turpentine, pine oils, rosin spirits and oils, alcohol, soap, etc. The total value of these products is greater than the value of the products of the destructive distillation of the wood.

The approximate comparative values of the products of the several chemical methods for the utilization of waste wood are indicated by the following table. The figures are values per cord of wood.

Good lightwood, destructively distilled	$25
Good lightwood, steam distilled	8
Good lightwood, soda extracted, and turpentine, rosin oils, and soda pulp made.	44
Ordinary long-leaf pine, soda extracted, and soda pulp	35
Good lightwood, extracted with volatile solvent and turpentine, rosin and soda pulp made	40

That is, the values obtained from a cord of wood by making paper pulp are from $10 to $37 greater than when the wood is destructively distilled or steam distilled and the wood burned or thrown away.

INDUSTRIAL DEVELOPMENT.

It is believed that the utilization of waste woods for the recovery and manufacture of wood turpentine, wood oils, rosin, rosin spirits, and oils, tars, pitches, acetates, alcohol, and paper pulp can be made a profitable chemical industry.

This, of course, does not mean that any ill-considered scheme of utilizing this material will prove profitable. On the contrary, only the best and most thoroughly developed plans, embracing all those considerations which are the foundation of well-organized industries, can be expected to reap the greatest returns from the opportunities which as yet lie practically undeveloped. It is not proposed to discuss here the details of equipment, arrangement of plant, or operation and technique of the various processes employed. The chemical utilization of wood in the ways suggested requires the adaptation and coordination of the equipment and processes which have been employed successfully in other industries. This is a comparatively simple problem for the experienced chemical engineer, but is not within the scope of this publication.

There are a number of ways in which the machinery for the manufacture of pulp and other products may be assembled, and many forms of apparatus may be employed. Some general suggestions, however, may be made. The observations and experiments of the bureau fully warrant the conclusion that a rotary digester will give larger yields of crude oils at less expense than will fixed retorts. It is also best to use a rotary digester in pulp making. The greater yield of oils with the rotary digester is due to the more rapid and complete penetration of the chips by the steam. Hand labor should be reduced to a minimum by the use of machinery. The arrangement of the plant should be such that repeated and useless handling of material is avoided and the products should be carried to the ultimate form in which they find industrial application. Incoming wood should be unloaded directly into the chipper and only the excess stored. Indeed there should be as little storage of wood as possible. It is far better that the wood should be brought daily to the works as soon as it is cut and split in order to avoid the great loss of turpentine which occurs at the surface of freshly cut and split pine.

Such a plant should be managed by a man skilled in its technical control in order that an accurate knowledge may be had of the quality and quantity of the raw material used and of the products obtained therefrom. Undoubtedly much of the dissatisfaction which has existed with regard to wood turpentine has been occasioned by the ignorance of the producer of the properties which the material should possess, or of the methods of producing a turpentine uniform in composition and resembling closely gum turpentine.

The wood should be cut diagonally across the grain and chipped to a fairly uniform size. The chips should not be more than three-fourths of an inch through nor less than one-fourth of an inch and may be subsequently shredded with advantage. Great care should be exercised to avoid the production of dust on the one hand and large slivers or chunks on the other. Very fine material loses turpentine before it reaches the digester, and is overcooked, resulting in low yields of turpentine and of weak pulp. Penetration of the large chips by steam or alkali is very slow and often incomplete, and consequently the time of steaming and digestion is prolonged, the yield of turpentine is lowered and the pulp is insufficiently cooked, which results in inferior pulp and paper.

The bureau has found in laboratory experiments that when the wood is prepared to pass a ¼-inch screen the oils are removed completely in less than two hours steaming and no more is obtained on cooking. When prepared to pass an inch screen, but not a ¼-inch screen, the oils are removed in from two to two and one-fourth hours steaming, and no more is obtained on further cooking. If the chips will pass a 2-inch screen, but not an inch screen, it requires three hours steaming and 12 per cent of the total oils is obtained in subsequent cooking. With properly prepared wood, the time for removal of turpentine is reduced at least one-third. The advantages of reducing the wood to a proper size and using a rotary have been demonstrated in the laboratory; 0.25 to 0.88 per cent of oils were recovered from the chips which had been steamed in a fixed retort. The smallest recovery was from chips passing a ¼-inch screen and the largest amounts from chips not passing a 1-inch screen. The chips should, therefore, be screened before they are placed in the digester and those which are too large should be made smaller by regrinding, or, preferably, by shredding.

Under proper conditions the turpentine can be steamed out of the chips in about three hours. So far as the removal of the turpentine is concerned, only a small pressure need be carried on the rotary until the crude oils are off; 40 pounds pressure applied intermittently is ample. The pressure may then be raised for the purpose of cooking the wood. The wood should not be reduced to chips and stored as such for any length of time before steaming, as cut-up wood loses turpentine quite rapidly, especially in warm weather. (Hogged wood has been known to lose half the turpentine in one week.) If the grinding plant can not deliver the chips to the digester with sufficient rapidity, chipping may be begun before the digester is ready to receive the charge, and the chips held for a short time in an overhead bin, from which they can be spouted to the digester. Large amounts of chips should not be so stored, however, and losses due to volatilization or fire must be guarded against.

Two digesters, each holding 8 cords (12 to 16 tons) of chips, can handle at least 50 cords (75 to 100 tons) of wood per 24 hours, three cooks being made in each digester. If lightwood is used, from 400 to 1,000 gallons of crude oils should be obtained by steaming the chips. From the black liquor by destructive distillation, from 1,500 to 5,000 gallons of oils, consisting of rosin spirits and rosin oils mixed with oils (chiefly phenols and cresols) derived from the ligneous material removed by the soda, are obtained. The full amount of methyl alcohol usually obtained by destructive distillation of pine wood, together with traces of acetic acid, are also contained in the aqueous distillate from the black liquor. From the residue of the distillation of the black liquor, sodium carbonate is dissolved and recausticised in the usual way.

Preferably a globe-shaped digester revolved on hollow trunnions should be used. The cooking liquor and steam are fed through one trunnion while the steam carrying the volatile oils is removed through the other. When the oils practically cease to come over the valve in the take-off trunnion is closed and the wood cooked at the proper pressure.

Suitable equipment for a plant making steam wood turpentine and using 50 cords of wood per day consists of two 8-cord rotary digesters, a total boiler capacity of 450 horsepower, engine capacity of 250 horsepower, a hog, a shredder, conveyers, condensers, refining stills, storage tanks, pumps, piping, and building. The whole will cost between $35,000 and $50,000.

REFINING.

Steam and crude oil vapors should not be condensed together before refining, as this calls for unnecessary apparatus and the increased steam consumption makes the operating expenses larger. The vapors should be so handled as to separate the light oil with some heavy oil from most of the heavy oils and the water. For refining 1,000 or more gallons of crude oils per day a continuous column still should be used, large enough to take care of the volume of steam entering it, and so constructed that two, and possibly three, distinct products may be obtained. Each column should be so arranged that its contents can not be carried forward to the column containing the lighter oils, but the residues from the fractionation in one column may pass to the next lower one from which heavier oils are obtained and so on until the condensed water is practically freed from oils and goes to waste from the bottom of the last column. Stills of this character can be built, and while their first cost is high, they insure, when properly constructed and operated, the separation of fairly uniform products.

The experiments made in this laboratory also show that in order to obtain a wood turpentine, which will comply in specific gravity, refractive index, evaporation, and behavior on distillation with gum spirits, it is necessary that the heavier oils be removed from contact with live steam as rapidly and as completely as possible. To this end the fractionating plates should carry the minimum depth of liquid and the steam should not be allowed to continue to pass through the heavy oils after they have been freed from the light oils. The hot heavy oils pass downward to other fractionating chambers, each of which may be operated to yield a fairly definite product, or they may be refined in a pot still. The general character of the products obtained with the column still is shown in Tables 4 to 8, pages 62 to 66.

In these plants when smaller quantities of crude oils are to be refined, the first cost of the columns and difficulties of control make the use of two simpler stills advisable, a periodic column still for the turpentine and a simple gooseneck still for the refining of the heavy or pine oils. The same apparatus is suitable for the refining of the oils obtained by the destructive processes. It is necessary to wash these products with soda or lime, as has been pointed out, and also to refractionate once, or even several times, the portions obtained from the first or preliminary fractionation.

As long as the distillate contains only water and such oils as may properly be termed wood turpentine the percentage of wood turpentine in the distillate will be from 62 to 54 per cent and throughout nearly the whole distillation this relation may exist. When large quantities of pine oils begin to distill, however, the ratio of oil to water in the distillate drops rapidly and the changing quantity of oil in the distillate, together with the speed with which the ratio of oil to water changes, furnishes the best evidence of the nature of the distilling oils. It has been observed in general that when a column still is employed and the percentage of oils in the distillate decreases rapidly, the oils may be mixed with the turpentine until the percentage of oils in the final distillate falls to about 45 per cent. If a pot still is employed the indications are that when the percentage of oil in the distillate falls below 50 it should no longer be mixed with the turpentine, since distillates containing less than these amounts no longer meet the requirements for turpentine and should be run to another tank until the percentage of oil in the distillate falls below 30 per cent, when the distillate should be run into a third tank. The oils in the third tank will consist of pine oils alone, while those in the second tank will consist of a mixture of turpentine and pine oils and should be again distilled, and the distillate run into tank 1 until the oil in the distillate falls below 50 per cent, when the remainder is run into tank 3 without further distillation.

STANDARDS OF QUALITY.

Since wood turpentine is used as a substitute for gum spirits of turpentine, it should comply with the customary tests applied to gum spirits to determine its suitability for this purpose and also with specifications for gum spirits.

Inasmuch as the highest grade of wood turpentine need not be employed in certain kinds of work, several grades, each suitable for a specific purpose, may be made. In this way users will be enabled to buy economically such wood turpentine as is suitable for their various purposes, the utilization of the heavier oils will be promoted, and the less perfectly equipped and operated plants will be able to find a market for their products.

Three grades have been suggested by this bureau for gum spirits,[1] and there appears to be no reason why wood turpentines should not be graded in the same way and comply with the same specifications. These specifications are:

Standard or No. 1 turpentine should have a specific gravity at 20° C. of from 0.862 to 0.870; a refractive index at 20° C. of from 1.468 to 1.476; 95 per cent should distill below 170° C., and a layer of not less than 200 mm should be required to equal in color the Lovibond yellow glass No. 1. On polymerization with 38 normal sulphuric acid the residue should not exceed 1 per cent, should be reddish in color and viscous, and its refractive index at 20° C. should be from 1.500 to 1.520. An unadulterated turpentine which does not agrée with these requirements may properly be regarded as not of standard or No. 1 quality.

Second quality or No. 2 turpentine should have a specific gravity at 20° C. of from 0.862 to 0.875; a refractive index at 20° C. of from 1.468 to 1.480; 90 per cent should distill below 170° C. and a depth of not less than 100 mm should be required to equal the Lovibond yellow glass No. 1. The polymerization residue must not exceed 1 per cent and must have a refractive index of not less than 1.50.

Third quality or No. 3 turpentine should have a specific gravity at 20° C. of from 0.865 to 0.880; a refractive index at 20° C. of from 1.468 to 1.485; 60 per cent should distill below 170° C. and a depth of not less than 50 mm should be required to equal the Lovibond yellow glass No. 1. The polymerization residue must not exceed 1 per cent and must have a refractive index of not less than 1.500.

Wood turpentine that complies with the specifications for No. 1 and No. 2 turpentines will have but little objectionable odor, and it is believed will meet all reasonable technical requirements for paint and varnish thinners, except possibly those for varnishes of the highest grade.

VALUE OF WOOD TURPENTINES AS PAINT AND VARNISH THINNERS.

The experimental work which has been done and the information that has been collected leave no doubt that wood turpentine, both destructive and steam distilled, can, by careful refining, be made to correspond closely with gum spirits in all those properties by which the suitability of turpentine, as a paint and varnish thinner, is judged. The wood turpentine that contains no products of destructive distillation and is free from heavy oils differs very slightly in odor from gum spirits, and but little, if any, objection can be raised to the technical use of well-refined wood turpentine on this account. It may be used in well-ventilated places without serious inconvenience to the workmen. Well-refined wood turpentine is a suitable paint and varnish material, except possibly for the highest grade varnishes; and it may be employed for these purposes without detriment to the paint or varnish.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 135.

TABULATION OF RESULTS.

TABLE 1.—*Commercial and experimental samples of refined wood turpentine.*

STEAM DISTILLED.

Laboratory No.	Place of sampling.	Date of sampling.	Process.	Sampled by—	Remarks.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—			Per cent residue above 185° C.	Specific rotation at 20° C.	Color.		Iodin absorption.			Saponification number.	Acidity number.
									160° C.	170° C.	185° C.			Yellow.	Red.	Total.	Addition.	Substitution.		
								°C.												
29	Lyons, Ga.	1904	Bethune	W. C. Douglas		0.8690	1.4685	....	....	....	....	....	+16.6	....	....	408.8	316.8	46.0	2.5	0.0133
58	do	June, 1904				.8740	1.4699	....	....	....	....	....	....	....	....	....	....	....	5.2	.0356
1174	Waycross, Ga.	Nov., 1905	Krug	F. P. Veitch		.8710	1.4	156	26	74	86	14	+19.2	5.0	0.2	362.7	294.3	34.2	7.7	.0270
1177	Tallahassee, Fla.	do		M. G. Donk		.8680	1.4683	155	50	89	97	3	+19.5	10.0	1.4	417.0	313.4	52.0	3.2	.0358
1178	do	do		do	Filtered through bone black.	.8700	1.4684	158	42	88	95	5	+19.8	1.7	.0	422.5	303.3	59.6	3.2	.0438
1179	do	do		do	Steam refined	.8660	1.4673	155	67	93	98	2	+20.9	.5	.0	427.5	328.5	49.5	1.9	.172
1180	Lyons, Ga.	do		F. P. Veitch		.8750	1.4699	156	44	85	92	8	+15.3	.7	.0	413.5	291.7	60.9	6.1	.0319
1186	Burnt Fort, Ga.	Sept., 1906		M. G. Donk		.8640	1.4675	157	45	91	98	2	+17.9	.5	.0	403.5	301.9	55.8	1.5	.0138
1187	Georgetown, S. C.	do		C. E. Sholes & Co.		.8770	1.4700	155	28	76	85	15	+18.6	1.1	.0	339.0	246.2	46.9	9.8	.0165
1188	do	do		do		.8700	1.4682	157	45	88	95	5	+20.1	.8	.0	403.4	294.2	54.6	4.0	.0135
1189	do	do		do		.8660	1.4696	156	59	94	98	2	+ 9.5	1.2	.0	423.5	279.3	57.1	3.7	.0320
1191	Valdosta, Ga.	Nov., 1905	Pittman (?)	Manufacturer		.8820	1.4707	154	25	74	85	15	+20.5	....	....	390.7	289.3	50.7	12.3	.0430
1193	Wilmington, N. C.	do	Weed	Magnus & Lauer		.8760	1.4697	155	40	82	91	9	+19.7	1.3	.0	395.8	279.0	58.4	9.0	.0115
1227	Lyons, Ga.	do		F. P. Veitch		.8680	1.4691	158	36	87	95	5	+15.3	1.5	.0	408.5	309.5	49.5	3.0	.0348
1235	Blakely, Ga.	1906	Krug	Manufacturer		.9150	1.4754	155	30	56	72	28	+18.3	....	....	350.1	195.3	77.4	23.8	.2447
1236	Lyons, Ga.	do	Bethune	T. A. Scarboro		.8650	1.4685	153	72	93	96	4	+16.4	.5	.0	430.0	313.2	58.4	3.2	.0113
1348	South Brewer, Me.	Nov., 1906	Sulphite	Manufacturer	From spruce wood	.8610	1.4876	172	....	....	99	1	+ 0.2	.7	.0	106.3	34.3	35.5	0.5	.0018
1539	Live Oak, Fla.	Feb., 1906	Bethune	F. P. Veitch		.9500	1.4816	153	36	48	62	38	+ 9.1	8.5	.3	338.7	219.5	59.6	44.1	.1293
1557	do	do	Snell	do		.8790	1.4706	153	62	79	87	13	+12.7	1.5	.2	417.2	235.6	55.8	6.6	.0253
1564	Wilmington, N. C.	Apr., 1906	Weed	Manufacturer		.8590	1.4689	154	73	93	97	3	+18.6	.7	.0	426.2	312.0	57.1	1.6	.0018
1813	Mississippi	May, 1907	Kerr	do		.8620	1.4683	153	84	92	96	4	+24.2	....	....	425.0	336.2	44.4	3.4	.0091
2655	Orange, Tex.	Aug., 1907		do		.8620	1.4676	152	88	95	95	5	+22.1	....	....	431.3	329.9	50.7	1.3	.0024
2656	do	do		do	Distilled, deodorized, and redistilled.	.8620	1.4678	152	85	93	96	4	+23.8	....	....	469.3	304.5	82.4	1.7	.0065
10949		Jan., 1910		Dealer		.8660	1.4680	158	[1] 43	69	86	14	....	....	....	....	....	....	....	....
10950		do		do		.8991	1.4755	177	....	....	20	80	....	....	....	....	....	....	....	....
11147	Waycross, Ga., Savannah yards.	Oct., 1909		F. P. Veitch		.8777	1.4698	151	14	69	[2] 80	[3] 20	....	....	....	....	....	....	....	....
14943	Branford, Fla.	1910		Manufacturer		.8647	1.4675	158	10	81	[2] 88	[3] 12	....	....	....	....	....	....	....	....
15057	Georgetown, S. C.	Jan., 1911		Dr. Teeple		.8622	1.4675	157	57	95	....	[4] 5	....	....	....	....	....	....	....	....

DESTRUCTIVELY DISTILLED.

59	Crichton, Ala.	Dec., 1904.	Mallonee	Manufacturer		0.8690	1.4688	157	47	79	89	11	+18.1	....	....	384.8	301.4	41.7	9.4	0.0098
62	Mobile, Ala.	Jan., 1905.	do	do		.8650	1.4696	157	11	50	70	30	+13.7	....	....	380.5	271.5	54.5	4.9	.0081
1175	Fayetteville, N. C.	Nov., 1905.	Clark	F. P. Veitch		.8600	1.4000	154	64	92	94	6	+ 6.1	1.6	0.0	398.4	322.2	38.1	3.9	.0110
1176	Savannah, Ga.	do	Mallonee	do		.8570	1.4723	162	....	66	96	4	+ 9.6	.8	.0	359.9	282.9	35.5	0.9	.0040
1184	Jacksonville, Fla.	do	McKeithan	Manufacturer		.8660	1.4676	154	52	88	94	6	+21.8	1.0	.0	414.9	310.9	52.0	1.9	.2330
1195	Philadelphia, Pa.	do		C. Schrank & Co.		.8630	1.4691	154	42	88	94	6	+19.9	(5)	(5)	(5)	(5)	(5)	(5)	(5)
1229	Jacksonville, Fla.	do	McKeithan	F. P. Veitch		.8740	1.4718	150	21	76	89	11	+17.2	4.5	.8	369.2	275.4	46.9	6.1	.0346
1237	Vancouver, British Columbia.	1906.		Bray	Douglas fir	.8640	1.4695	153	30	52	68	32	−50.4	12.0	1.5	343.8	247.4	48.2	7.5	.0277
1238	do	do		Manager	do	.8620	1.4666	156	70	93	97	3	−44.9	.4	.0	436.1	323.3	55.9	2.1	.0143
1317	Savannah, Ga.	Dec., 1906.	Mallonee	Manufacturer	Washed with soda and distilled.	.8880	1.4746	....	....	....	....	....	+ 6.5	....	....	273.5	183.5	45.0	17.3	.0533
1318	New York	1906.		do	Treated to deodorize.	.9230	1.4790	158	7	49	65	35	+16.6	....	....	340.4	259.2	40.6	....	....
1319	Kipling, Ala.	do		do		.8930	1.4692	....	....	....	....	....	....	....	....	322.2	247.0	37.6	1.6	.0026
1542	Albany, Ga.	Feb., 1906.	Bilfing	F. P. Veitch	No. 1	.8980	1.4742	154	51	71	83	17	+16.9	5.0	3.0	375.5	258.7	58.4	19.0	.2343
1543	do	do	do	do	No. 2	.8940	1.4791	120	41	68	87	13	(6)	....	....	292.7	159.9	66.5	(7)	(7)
1561	Fayetteville, N. C.	do	Clark	do		.8760	1.4680	152	79	88	93	7	+19.7	.8	.0	418.6	312.0	53.3	7.9	.0128
10951	New York	Jan., 1910.		Dealer		.8678	1.4810	169	....	....	61	39	....	....	....	....	....	....	....	....

[1] Below 165° C.
[2] Below 180° C.
[3] Above 180° C.
[4] Above 175° C.
[5] Missing.
[6] Too dark.
[7] No end point.

TABLE 2.—*Pine oils and still residues.*

STEAM DISTILLED.

Laboratory No.	Description of sample.	Date of sampling.	Source.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilled below—						Color.		Iodin value.		Saponification number.	Acidity number.
							185° C.	200° C.	215° C.	225° C.	240° C.	250° C.	Yellow.	Red.	Addition.	Substitution.		
						° C.												
1181	First heavy oil distillate.	Nov., 1905	Waycross, Ga.	0.889	1.4783	170	26	64	92	97	......	......	1.7	0	178.9	48.2	28.0	0.0078
1182	Second heavy oil distillate.	do.	do.	.932	1.4844	[1] 102	[1] 2	6	64	95	......	......	2.5	0	92.6	53.3	2.7	.0042
1363	Refining still residue after No. 1182.	do.	do.	.927	......	[1] 99	0	0	[1] 1	45	45	70	......	......	96.4	49.5	1.5	([2])
1366	No. 2 white oil	do.	Lyons, Ga.	.928	......	102	[1] 2	29	93	96	......	......	2.6	0	129.3	62.2	3.9	.0339
1367	Refining still residue	do.	do.	.960	......	98	0	......	[1] 1	[3] 18	28	......	......	......	119.3	67.2	14.6	([2])
1540	Light oil	Feb., 1906	Live Oak, Fla.	.937	1.4817	100	......	[1] 6	89	......	......	......	30.0	5.0	121.7	49.5	6.5	.0449
1541	Heavy oil after light oil.	do.	do.	.951	1.4894	190	......	11	56	71	85	......	......	......	122.5	58.4	([4])	([4])
2657	Light oil tailings from refining still.	Aug., 1907	Orange, Tex.	.944	1.4880	100	[1] 4	22	90	......	......	......	......	......	126.0	45.0	4.5	.0386
2658	Heavy oil tailings from refining still.	do.	do.	.958	1.4975	205	......	......	52	68	......	......	......	......	91.3	77.4	([4])	.0177
1565	Refined pine oil	Apr., 1907	Wilmington, N. C.	.937	1.4844	±100	[1] 2	31	86	90	......	......	20.0	2.8	118.0	52.0	11.5	.2071
1814	Refined pine oil (soda extracted).	May, 1907	Lynchburg, Va.	.944	1.4855	100	[1] 3	20	94	......	......	......	......	......	148.0	52.0	9.2	.1783
1815	Crude pine oil (soda extracted).	do.	do.	.931	1.4852	180	4	32	76	88	[5] 93	[6] 181	......	......	156.1	55.8	([4])	.0392
14944	Water-white pine oil	1910	Branford, Fla.	.8942	1.4765	173	[7] 51	[8] 130	......	......	......	......	......	......	......	......	......	......
14945	Straw-colored pine oil	do.	do.	.9434	1.4819	210	......	......	38	[9] 117	......	......	[10] 34 0	......	......	......	......	......
DESTRUCTIVELY DISTILLED.																		
44	Pine oil	Nov., 1905	Jacksonville, Fla.	0.910	1.4864	153	61	72	78	84	......	......	......	......	......	......	([4])	([4])
1183	do.	do.	Savannah, Ga.	.907	1.4344	162	23	......	......	......	......	......	15.5	2.0	166.2	59.6	6.0	0.0026
1550	Crude heavy oil	Feb., 1906	Collins, Ga.	1.035	1.5520	152	......	10	10	10	24	......	......	......	13.8	111.5	([4])	([4])
1544	do.	do.	Albany, Ga.	.958	1.5028	140	......	48	58	62	68	75	......	......	75.1	101.1	([4])	([4])

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[1] Contained water.
[2] Too dark.
[3] Below 230°.
[4] No end point.
[5] Temperature fell from 130½°.
[6] Below 130½°.
[7] Below 180°=32 per cent.
[8] Below 205°.
[9] Below 220°.
[10] Depth in millimeters to equal Lovibond glass No. 1 yellow.

TABLE 3.—*Crude wood turpentine.*

STEAM DISTILLED.

Laboratory No.	Process.	Place of sampling.	Date of sampling.	Sampled by—	Remarks.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—			Per cent residue above 185° C.	Specific rotation as 20° C.	Color.		Iodin absorption.			Saponification number.	Acidity number.
								° *C.*	160° C.	170° C.	185° C.			Yellow.	Red.	Total.	Addition.	Substitution.		
1177	......	Tallahassee, Fla.	Nov., 1905	Manufacturer	......	0.8680	1.4683	155	50	89	97	3	+19.5	10.0	1.4	417.4	313.4	52.0	3.2	0.0359
1194	Bennor	Macon, Ga	do	do	......	.8790	1 4719	155	21	74	85	15	+15.2	35.0	9.8	......	......	......	8.9	.1610
1227	do	do	do	......	......	.8680	1.4691	158	36	87	95	5	+15.3	1.5	.0	408.5	309.5	49.5	3.0	.0248
1228	......	Waycross, Ga	do	F. P. Veitch	......	.8840	1.4733	156	20	64	80	20	+15.0	......	......	431.3	322.3	54.5	2.6	.0235
1360	Bennor	Macon, Ga	Feb., 1906	do	......	.8710	1.4711	157	12	75	89	11	+22.2	13.5	1.8	407.2	285.4	60.9	3.2	.0077
1365	Bethune	Lyons, Ga	do	do	......	.8740	1.4720	152	13	71	88	12	+12.5	1.1	.0	398.3	281.5	58.4	2.6	.0154
1538	......	Live Oak, Fla	do	do	......	.8760	1.4932	153	66	85	91	9	+12.8	......	......	412.3	313.3	49.5	3.0	.0177
1552	......	Macon, Ga	do	do	First running.	.8660	1.4686	151	77	89	95	5	+17.9	24.0	5.0	425.0	313.4	55.8	2.6	.0424
1553	......	do	do	do	Middle running.	.8730	1.4612	152	67	81	89	11	+23.2	12.0	15.0	408.6	284.2	62.2	7.3	.1157
1555	......	do	do	do	Late running; kept under pressure one-half hour.	.8920	1.4713	......	......	......	......	......	+10.0	......	......	386.3	273.3	56.5	10.8	0314
1556	......	Live Oak, Fla	do	do	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......
1558	......	Wilmington, N. C.	do	do	......	.8850	1.4741	......	......	......	......	......	+ 9.3	95.0	20.5	390.7	279.1	55.8	5.9	.1268
1556	Weed	do	do	Manufacturer	......	.9370	1.5059	160	0	6	24	76	(1)	21.6	6.0	386.3	273.3	56.5	8.8	.2155
2654	......	Orange, Tex	Aug., 1907	do	......	.8760	1.4733	154	47	74	84	16	+20.4	......	......	386.9	280.3	53.3	2.5	.0083
15051	Steam and H_2SO_4.	Georgetown, S. C.	Jan., 1911	do	......	.8724	1.4748	165	......	20	78	22	......	......	......	......	......	......	......	......
15052	Steam and soda.	De Soto, Miss	Apr., 1909	do	......	.8900	1.4728	160	[2] 15	40	72	28	......	......	......	......	......	......	......	......
20534	......	Branford, Fla	——, 1911	do	......	.8967	1.4730	......	......	32	68	32	......	......	......	......	......	......	......	......

DESTRUCTIVELY DISTILLED.

Laboratory No.	Process.	Place of sampling.	Date of sampling.	Sampled by—	Remarks.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	160° C.	170° C.	185° C.	Per cent residue above 185° C.	Specific rotation as 20° C.	Yellow.	Red.	Total.	Addition.	Substitution.	Saponification number.	Acidity number.
1548	Mallonee	Collins, Ga	Feb., 1906	F. P. Veitch	Light oil No. 1.	0.9180	1.4915	150	21	46	67	33	(1)	......	......	315.0	215.0	50.0	(3)	(3)
1549	do	do	do	do	Light oil No. 2.	.9630	1.5107	145	10	19	34	66	(1)	......	......	286.6	104.2	91.2	(3)	(3)
1560	......	Fayetteville, N. C.	do	do	......	.8680	1.3319	152	76	89	91	9	+19.8	10.0	0.8	421.2	304.4	58.4	3.4	0.0323

[1] Too dark.

[2] Below 165°.

[3] No end point.

TABLE 4.—*Distillation data on refining crude steam-distilled wood turpentine with steam in a beer still.*

[Figures are on subsamples and total fractions.]

Barrel number and fraction.	Sample No.	Distillation in progress.	Temperature at top of still uncorrected.	Turpentine in fraction.	Turpentine in distillate.	Specific gravity at 20°C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilled below— 160° C.	165° C.	170° C.	175° C.	185° C.	200° C.	215° C.	230° C.
		Minutes.	*°C.*	*Gallons.*	*Per cent.*			*°C.*								
Crude turpentine						0.8964	1.4741	160		15	40	56	[1]66	[2]72	[3]77	[4]79
Crude turpentine						.8900	1.4728	158		11	37	53	[1]60	[2]64	[3]69	[4]72
Crude turpentine								160		15	40	57	70	84	94	
6A	1	0	96.4		63	.8618	1.4668	154	82	94	97		99			
6A	2	48	97.0		60	.8619	1.4682	156	56	85	93		99			
6A	3	62	97.5		54	.8635	1.4687	157	38	73	86		97	99		
6A	4	71	98.0		47	.8659	1.4699	158	4	59	76		94	98		
6A	5	75	98.2		46	.8669	1.4703	159	4	45	70		92	97		
6A	6	81	98.4		43	.8692	1.4717	160		31	57		83	90		
6A	7	84	98.7			.8734	1.4728	162		17	49		84	94	97	
6B	8	91	99.0		35	.8764	1.4738	±163		5	40		79	93		
6B	9	101	99.5		27	.8904	1.4765	±170			2		52	82	97	
6B	10	129	100.0			.9236	1.4809	±189						38	94	
6A	1 to 7	84	96.4 to 98.7	32½	52	.8627	1.4608	156	49	83	92		97			
6B	8 to 10	35	98.7 to 100.0	5	23	.8907	1.4776	±167			6		55	82	98	
						.9004	1.4775	165				12	42	69	85	

[1] Below 180°.

[2] Below 185°.

[3] Below 190°.

[4] Below 195°.

TABLE 5.—*Distillation data on refining crude steam-distilled wood turpentine in a column refining still.*

[Figures are on subsamples and total fractions.]

Barrel number and fraction.	Sample No.	Time of distillation.	Temperature.	Volume of turpentine.	Turpentine in distillate.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilled below—						
									160° C.	165° C.	170° C.	175° C.	185° C.	200° C.	215° C.
		Minutes.	*°C.*	*Gallons.*	*Per cent.*			*°C.*							
7A	1	2	96.8	......	62	0.8617	1.4669	155	74	92	96	98	......	......	......
7A	2	14	97.2	......	59	.8620	1.4671	156	52	......	94	97	98	......	......
7A	3	35	97.8	......	53	.8634	1.4679	157	28	75	87	92	96	97	......
7A	4	52	98.4	......	51	.8654	1.4691	158	1	51	76	86	93	97	......
7A	5	60	98.8	......	45	.8679	1.4700	159	......	19	57	76	91	96	......
7B	6	76	99.8	......	31	.8815	1.4745	165	......	......	6	24	64	88	......
7A	1 to 5	60	96.8 to 98.8	23	52	.8628	1.4674	156½	39	78	90	93	96	99	......
7B	6	10	98.8 to 99.6	3¾	35	.8740	1.4728	......	......	......	26	53	80	93	......
Residue No. 7	......	......	......	6	......	.8877	1.4751	163	......	......	17	43	68	83	......
8A	1	1	96.6	......	64	.8623	1.4669	155	59	88	96	97	98	......	......
8A	2	101	98.8	......	49	.8670	1.4702	159	......	37	64	79	91	97	......
8B	3	111	99.2	......	45	.8705	1.4715	161	......	4	38	63	86	95	......
8B	4	130	100.0	......	32	.8844	1.4717	170	......	......	......	13	55	87	......
8A	1 to 2	111	96.6 to 99.2	32½	52	.8630	1.4680	158	16	73	88	94	97	......	......
8B	3 to 4	19	99.2 to 100.0	3	32	.8748	1.4737	162	......	......	14	45	75	93	......
Residue No. 8	......	......	......	12	......	.9405	1.4820	......	......	......	......	......	......	......	......
9A	1	19	97.0	......	61	.8617	1.4685	155	64	88	94	96	97	......	......
9A	2	21	97.8	......	61	.8615	1.4673	155½	66	90	96	98	......	......	......
9B	3	152	98.8	......	43	.8715	......	164	......	2	36	60	83	......	......
9B	4	175	99.8	......	25	.8944	1.4786	±175	......	......	......	3	35	78	......
9A	1 to 2	152	96.4 to 98.8	32¼	50	.8635	......	156	32	77	89	93	96	......	......
9B	3 to 4	23	98.8 to 99.8	3½	32	.8809	1.4748	167	......	......	7	31	68	89	......
Residue No. 9	......	......	......	12¼	......	.9428	1.4810	......	......	......	......	......	......	......	......
10A	1 to 7	45	96.4 to 98.2	27	54	.8623	1.4680	156½	37	74	91	96	98½	......	......
10B	8 to 11	21	98.2 to 99.8	8	37	.8716	1.4720	160	......	8	48	68	83	92	......
10C	12	10	99.8 to 100.3	2¼	20	......	1.4788	173	......	......	......	1	23	68	......
10B+C	8 to 12	31	98.2 to 100.3	10¾	32	......	......	......	......	......	......	......	......	......	......
Residue No. 10	......	10½	......	......	......	.9450	1.4818	......	......	......	......	......	......	......	......

TABLE 6.—*Fractionation of crude steam-distilled wood turpentine in two forms of column stills.*

[Figures are for total fractions only.]

Barrel number and fraction.	Temperature at top of still.	Time required to distill fraction.	Turpentine in distillate.	Volume of fraction.	Turpentine recovered.[1]	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—					
									160° C.	165° C.	170° C.	175° C.	185° C.	200° C.
	°C.	*Minutes.*	*Per cent.*	*Gallons.*	*Per cent.*			*°C.*						
7 to 10A	95.4 to 96.0	181	59	40½	81	0.8600	1.4670	155½	81	96	98	......	......	......
7 to 10A+B	......	215	57	46	92	......	......	155½	......	88	95	......	......	......
1 to 6A	96.0 to 98.0	103	......	46½	93	.8607	1.4682	159 to 155	14 to 77	77 to 92	97	91 to 98	......	......
5A	96.8 to 97.6	56	56	19½	39	.8617	1.4677	155	42 to 74	84 to 90	92 to 95	......	......	......
10A+B	95.9 to 97.8	342	54	27	54	.8604	1.4687	156	34 to 56	83 to 89	91 to 96	......	......	......
11A	96.4 to 98.2	45	54	27	54	.8623	1.4680	156½	37	74	91	......	......	......
7A	96.8 to 98.8	60	52	23	69	.8628	1.4674	156½	39	78	90	......	......	......
8A	96.6 to 99.2	111	52	32½	65	.8630	1.4680	158	16	73	88	......	......	......
4A	96.0 to 98.6	140	52	32	64	.8688	1.4689	±157	15 to 48	71 to 78	85 to 90	......	......	......
6A	96.4 to 98.7	84	52	32½	65	.8627	1.4698	156	49	83	92	......	......	......
5A+B	96.8 to 99.0	87	51	29	58	......	......	155	......	72	85	......	......	......
3A	96.0 to 98.6	65	50	33½	67	.8637	1.4683	158	38	78	91	......	......	......
9A	96.4 to 98.8	152	50	32¼	64	.8637	......	156	32	77	89	......	......	......
7 to 10B+C	96.5 to 98.2	34	47	5¼	11	.8622	1.4703	163 to 158	0 to 0	27 to 34	66 to 73	80 to 87	88 to 97	......
7 to 10C	96.9 to 98.2	25	46	3¾	7	.8631	1.4710	161	......	13	63	82	95	......
5B	97.6 to 99.0	31	40	9½	19	.8700	1.4712	158	......	35	66	89	96	......
11B	98.2 to 99.8	21	37	8	16	.8716	1.4720	160	0	8	48	68	83	......
10C+D	97.8 to 99.7	130	36	5½	11	.8724	1.4705	163	......	......	27	66	80	......
7B	98.8 to 99.6	16	35	3¾	7	.8740	1.4728	163	0	0	17 to 26	43 to 53	68 to 80	......
8B	99.2 to 100.0	19	32	3	6	.8748	1.4737	162	0	......	14	45	75	......
9B	98.8 to 99.8	23	32	3½	7	.8809	1.4748	±167	......	......	7	31	68	......
6B	98.7 to 100.0	35	23	5	10	.8907	1.4776	165 to 167	......	......	6	12	42 to 55	......
5C	99.0 to 100.0	21	21	4	8	.8971	1.4777	±171	......	......	......	43	77	......
11C	99.8 to 100.3	10	20	2¼	4	......	1.4788	173	0	0	0	1	23	......
3B	98.6 to 99.0	75	18	10¼	21	.9111	1.4788	176 to 183	0	......	......	15	6 to 35	......
4B	98.6 to 100.0	117	14	12½	25	.9215	1.4788	......	......	......	......	......	7	......
1 to 6AB	98.0 to 100.0	19	......	4	8	.9137	1.4784	175	......	......	......	......	10	43
7 to 10A, still residue	......	......	......	¼	½	.9174	1.4785	......	......	......	......	......	19	53
5, still residue	......	......	......	17	34	......	......	......	......	......	......	......	......	......
10, still residue	......	......	......	11¾	23	.9489	1.4818	......	......	......	......	......	......	......
11, still residue	......	......	......	10½	24	.9450	1.4818	......	......	......	......	......	......	......
7, still residue	......	......	......	6	12	......	......	......	......	......	......	......	......	......
8, still residue	......	......	......	12	24	.9405	1.4820	......	......	......	......	......	......	......
4, still residue	......	......	......	5½	11	......	......	......	......	......	......	......	......	......
4, still residue	......	......	......	12¼	24	......	......	......	......	......	......	......	......	......
3, still residue	......	......	......	6¼	12	......	......	......	......	......	......	......	......	......
9, still residue	......	......	......	12¼	24	.9428	1.4810	......	......	......	......	......	......	......

[1] In most cases there was a loss of oil, either on the plate of the still or because of difficulty in emptying the still; therefore the recovery seldom equals 100 per cent.

TABLE 7.—*Redistillation of mixed first fractions.*

FROM BEER STILL.

Barrel number and fraction.	Sample No.	Distillation in progress.	Temperature at top of still uncorrected.	Turpentine in fraction.	Turpentine in distillate.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—						
									160° C.	165° C.	170° C.	175° C.	185° C.	200° C.	215° C.
		Minutes.	*° C.*	*Gallons.*	*Per cent.*			*° C.*							
Mixed first fraction from beer still						0.8610	1.4678	156 155	50 67	79 85	91 92	96 98	98		
1 to 6A	1	0	96.0		65	.8614	1.4667	153	87	96	98				
1 to 6A	2	2	96.0		61	.8601	1.4672	153	95	98					
1 to 6A	3	103	98.0		40	.8686	1.4737	163		9	49	69	89	97	99
1 to 6B	4	122	100.0		9	.9326	1.4790	185						17	85
1 to 6A	1 to 3	103	96.0 to 98.0	46.5		.8607 .8718	1.4682 1.4680	155 159	77 14	92 77	97	98 91			
1 to 6B	4	19	98.0 to 100.0	4.0		.9137	1.4784	175					10	43	83
FROM REFINING STILL.															
7 to 10A	1	2	[1] 95.8		58	0.8611	1.4665								
7 to 10A	2	6	95.6		59	.8601	1.4661								
7 to 10A	3	27	96.0		61	.8598	1.4662								
7 to 10A	4	61	95.4		61	.8601	1.4666								
7 to 10A	5	108	95.6		58	.8603	1.4669								
7 to 10A	6	181	96.5		54	.8599	1.4688								
7 to 10A	7	190	96.9		53	.8609	1.4698								
7 to 10A	8	215	98.2		39	.8669	1.4728								
A	1 to 6	181	95.4 to 96.5	40½	59	.8600	1.4670	155½	81	96	98				
B+C	7 to 8	34	96.5 to 98.2	5⅓	47	.8622	1.4703	158		34	73	87	97		
B+C						.8724	1.4705	163		27	66	80	88		
C	7 to 8	25	96.9 to 98.2	3¾	46	.8631	1.4710	161		13	63	82	95	99	
Residue						.9174	1.4787	172					19	53	75

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[1] Manipulating steam valve, cutting off steam all the time, and temperature falling.

TABLE 8.—*Summary of refining experiments with column stills showing the relation between the percentage of turpentine in the distillate and the composition of the turpentine.*

Sample.	Fractionated in—	Total time of distillation.	Time elapsed from beginning of distillation.	Temperature at top of column.	Turpentine in distillate.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.[1]	Per cent distilled below—		
									160° C.	165° C.	170° C.
		Minutes.	*Minutes.*	*° C.*	*Per cent.*			*° C.*			
1 to 6A 2	Beer still	122	2	96.0	61	0.8601	1.4672	153	....	98	99
1 to 6A 1	....do	122	0	96.0	65	.8614	1.4667	153	....	96	98
9A 2	Refining still	175	21	97.8	61	.8615	1.4673	155½	66	90	96
9A 1	....do	175	19	97.0	61	.8617	1.4685	155	64	88	94
7A 1	....do	76	2	96.8	62	.8617	1.4669	155	74	92	96
6A 1	Beer still	129	0	96.4	63	.8618	1.4668	154	82	94	97
6A 2	....do	129	48	97.0	60	.8619	1.4682	156	56	85	93
7A 2	Refining still	76	14	97.2	59	.8620	1.4671	156	52	....	94
8A 1	....do	130	1	96.6	64	.8623	1.4669	155	59	88	97
6A 3	....do	76	35	97.8	53	.8634	1.4679	157	28	75	87
5A 3	Beer still	129	62	97.5	54	.8635	1.4687	157	38	73	86
7A 4	....do	166	56	97.6	52	.8638	1.4693	157	29	72	87
7A 4	Refining still	76	52	98.4	51	.8654	1.4691	158	1	51	76
6A 4	Beer still	129	71	98.0	47	.8659	1.4699	158	4	59	76
6A 5	....do	129	75	98.2	46	.8669	1.4703	159	4	45	70
8A 2	Refining still	130	101	98.8	49	.8670	1.4702	159	0	37	64
7A 5	....do	76	60	98.8	45	.8679	1.4700	159	0	19	57
1 to 6A 3	Beer still	122	103	98.0	40	.8686	1.4737	163	0	9	49
6A 6	....do	129	81	98.4	43	.8692	1.4717	160	0	31	57
8B 3	Refining still	130	111	99.2	45	.8705	1.4715	161	0	4	38
9B 3	....do	175	152	98.8	43	.8715	....	164	0	2	38
5B 7	Beer still	166	76	98.5	42	.8717	1.4715	162	0	31	55
6A 7	....do	129	84	98.7	....	.8734	1.4728	162	0	17	49
6B 8	....do	129	91	99.0	35	.8764	1.4738	±163	0	5	40
6B 6	Refining still	76	76	99.8	31	.8815	1.4745	165	0	0	6
8B 4	....do	130	130	100.0	32	.8844	1.4717	170	0	0	0
6B 9	Beer still	129	101	99.5	27	.8904	1.4765	±170	0	0	0
9B 4	Refining still	175	175	99.8	25	.8944	1.4786	±175	0	0	0
6B 10	Beer still	129	129	100.0	....	.9236	1.4809	±189	0	0	0
1 to 6B 4	....do	122	122	100.0	9	.9326	1.4790	185	0	0	0

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[1] Emergent stem thermometer uncorrected.

TABLE 9.—*Refining experiments with a pot still, showing the percentage of turpentine in the distillate and the composition of the turpentine.*

FIRST RUN (total time of distillation, 900 minutes).

Sample No.	Time elapsed from beginning of distillation.	Turpentine in distillate.	Specific gravity at 20° C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—						
						160°C.	165°C.	170°C.	175°C.	180°C.	185°C.	190°C.
	Minutes.	*Per cent.*			°C.							
	0	14	......	......	......	......	......	......	......	......	......	......
	3	56	......	......	......	......	......	......	......	......	......	......
19923	4	54	0.8634	1.4660	153	30	79	90	92	93	......	97
	5	55	......	......	......	......	......	......	......	......	......	......
	10	53	......	......	......	......	......	......	......	......	......	......
	20	57	......	......	......	......	......	......	......	......	......	......
19924	45	54	.8638	1.4662	157	21	77	87	91	94	96	97
	100	53	......	......	......	......	......	......	......	......	......	......
	155	53	......	......	......	......	......	......	......	......	......	......
	215	50	......	......	......	......	......	......	......	......	......	......
	245	51	......	......	......	......	......	......	......	......	......	......
	255	51	......	......	......	......	......	......	......	......	......	......
	285	51	......	......	......	......	......	......	......	......	......	......
	300	51	......	......	......	......	......	......	......	......	......	......
	330	47	......	......	......	......	......	......	......	......	......	......
	335	47	......	......	......	......	......	......	......	......	......	......
	350	50	......	......	......	......	......	......	......	......	......	......
	360	51	......	......	......	......	......	......	......	......	......	......
	390	48	......	......	......	......	......	......	......	......	......	......
	410	47	......	......	......	......	......	......	......	......	......	......
	425	48	......	......	......	......	......	......	......	......	......	......
19925	495	45	.8665	1.4693	160	......	28	62	77	83	87	91
	505	44	......	......	......	......	......	......	......	......	......	......
	520	44	......	......	......	......	......	......	......	......	......	......
	530	44	......	......	......	......	......	......	......	......	......	......
	540	43	......	......	......	......	......	......	......	......	......	......
19926	555	43	.8686	1.4707	163	......	8	53	71	81	86	90
SECOND RUN (time of distillation, 800 minutes).												
	300	55	......	......	......	......	......	......	......	......	......	......
	385	51	......	......	......	......	......	......	......	......	......	......
	460	50	......	......	......	......	......	......	......	......	......	......
	505	50	......	......	......	......	......	......	......	......	......	......
	520	49	......	......	......	......	......	......	......	......	......	......
	570	51	......	......	......	......	......	......	......	......	......	......
	580	44	......	......	......	......	......	......	......	......	......	......
19927	620	40	0.8693	1.4710	161	......	5	51	69	76	85	88
	650	33	......	......	......	......	......	......	......	......	......	......
	675	37	......	......	......	......	......	......	......	......	......	......
19920	720	30	.8811	1.4750	166	......	......	3	10	41	60	70
19921	745	27	.8868	1.4753	173	......	......	......	9	20	45	62
THIRD RUN (time of distillation, 835 minutes).												
	345	53	......	......	......	......	......	......	......	......	......	......
	435	52	......	......	......	......	......	......	......	......	......	......
	525	49	......	......	......	......	......	......	......	......	......	......
	545	47	......	......	......	......	......	......	......	......	......	......
19914	565	44	0.8683	1.4690	162.0	......	16	59	75	82	86	91
19915	610	38	.8718	1.4715	163.5	......	4	36	61	74	80	88
	625	38	......	......	......	......	......	......	......	......	......	......
19916	655	33	.8763	1.4740	167.0	......	......	7	36	62	70	78
	670	32	......	......	......	......	......	......	......	......	......	......
19917	685	27	.8830	1.4750	166.0	......	......	3	12	40	55	74
19918	715	26	.8889	1.4770	171.0	......	......	......	6	11	44	60
19919	760	21	.9005	1.4790	176.0	......	......	......	......	1	4	32
	775	20	......	......	......	......	......	......	......	......	......	......
	795	20	......	......	......	......	......	......	......	......	......	......
19922	810	18	.9140	1.4792	183.0	......	......	......	......	......	4	7
FOURTH RUN (time of distillation, 720 minutes).												
	15	59	0.8628	......	......	......	......	......	......	......	......	......
	18	55	......	......	......	......	......	......	......	......	......	......
	22	57	.8621	......	......	......	......	......	......	......	......	......
	25	54	......	......	......	......	......	......	......	......	......	......
	147	56	.8619	......	......	......	......	......	......	......	......	......
	152	57	......	......	......	......	......	......	......	......	......	......
	157	58	.8609	......	......	......	......	......	......	......	......	......

TABLE 10.—*Summary of Table 9 showing constants of turpentine samples taken during the progress of distillation, and the percentage of turpentine in the distillate.*

[Total time for distillation of turpentine No. 1, 900 minutes; No. 2, 800 minutes; No. 3, 835 minutes.]

Sample number.	Distillation number.	Time elapsed from beginning of distillation.	Turpentine in distillate.	Specific gravity at 20°C.	Refractive index at 20° C.	Initial distilling temperature.	Per cent distilling below—						
							160° C.	165° C.	170° C.	175° C.	180° C.	185° C.	190° C.
		Minutes.	*P. ct.*			° *C.*							
19923	1	4	54	0.8634	1.4660	153	30	79	90	92	93	.....	97
19924	1	45	53-54	.8638	1.4662	157	21	77	87	91	94	96	97
19925	1	495	45	.8665	1.4693	160	0	28	62	77	83	87	91
19914	3	565	44	.8683	1.4690	162	0	16	59	75	82	86	91
19926	1	555	43	.8686	1.4707	163	0	8	53	71	81	86	90
19927	2	620	40	.8693	1.4710	161	0	5	51	69	76	85	88
19915	3	540	38-39	.8718	1.4715	163½	0	4	36	61	74	80	88
19916	3	630	33	.8763	1.4740	167	0	0	7	36	62	70	78
19920	2	720	30	.8811	1.4750	166	0	0	3	10	41	60	70
19917	3	685	27	.8830	1.4750	166	0	0	3	12	40	55	74
19921	2	745	27	.8868	1.4753	173	0	0	0	9	20	45	62
19918	3	720	25	.8889	1.4770	171	0	0	0	6	11	44	60
19919	3	765	22	.9005	1.4790	176	0	0	0	0	1	4	32
19922	3	810	18	.9140	1.4792	183	0	0	0	0	0	4	7

TABLE 11.—*Detailed opinions of the workmen on the varnishes used.*

PIANO FINISHING OR FLOWING VARNISHES.

Data.	Varnish.	A. B. Chase Co.	Wm. Knabe & Co.	Murphy Varnish Co.	Devoe & Raynolds Co.	Steinway & Co.	American Paint Manufacturing Association.
Varnishes were thinned with—	1. Gum spirits	Not thinned	Not thinned	1 part thinner to 13 parts varnish.	2 parts thinner to 1 part varnish.	All thinned alike. Coat No. 1, 1 part turpentine to 3.3 parts varnish. Coat No. 2, 1 part turpentine to 5 parts varnish.	All thinned alike (120 cc varnish to 30 parts turpentine).
	2. Steam distilled spirits.			1 part thinner to 9 parts varnish.	Not thinned		
	3. Destructively distilled spirits.			Not thinned	do		
	4. Destructively distilled spirits.			Not thinned (too thin).	do		
	5. Gum spirits (probably).			1 part thinner to 32 parts varnish.	do		
Varnishes remained tacky.	1. Gum spirits	About 4 hours	Still tacky in 8½ hours	More than 24 hours.	Average time 48 hours for all.	About 50 to 55 hours.	7 hours 25 minutes.
	2. Steam distilled spirits.	do	do	24 hours		do	7 hours 15 minutes.
	3. Destructively distilled spirits.	do	do	do		do	7 hours 10 minutes.
	4. Destructively distilled spirits.	do	7½ hours	20 hours		do	7 hours 45 minutes.
	5. Gum spirits (probably).	About 5 hours	Still tacky in 8½ hours	24 hours		About 45 to 48 hours.	7 hours.
Varnishes hardened in	1. Gum spirits	Allowed 1 week between coats.	About 15 days	3 days	Average time 72 hours.	All in about 1 month.	38 hours.
	2. Steam distilled spirits.						Do.
	3. Destructively distilled spirits.						37 hours.
	4. Destructively distilled spirits.						38 hours.
	5. Gum spirits (probably).						Do.
Varnish was applied in—	1. Gum spirits	Two coats	One coat	Two coats	Three coats	Two coats	Six coats.
	2. Steam distilled spirits.						
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.						
	5. Gum spirits (probably).						

TABLE 11.—*Detailed opinions of the workmen on the varnishes used*—Continued.

PIANO FINISHING OR FLOWING VARNISHES—Continued.

Data.	Varnish.	A. B. Chase Co.	Wm. Knabe & Co.	Murphy Varnish Co.	Devoe & Raynolds Co.	Steinway & Co.	American Paint Manufacturing Association.
Gloss of varnish surface.	1. Gum spirits	All alike	All alike	Poorest gloss		Medium gloss varnishes.	All good (1 and 3 show tears).
	2. Steam distilled spirits.						
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.						
	5. Gum spirits (probably).						
Odor is objectionable.	1. Gum spirits	Objectionable	Not objectionable	Old fatty oil odor	Not objectionable	Not objectionable	
	2. Steam distilled spirits.		do.	Odor like turpentine substitute.	Objectionable	Objectionable	
	3. Destructively distilled spirits.		Objectionable	do.	Not objectionable	Not objectionable	Objectionable.
	4. Destructively distilled spirits.		do.	do.	do.	do.	Do.
	5. Gum spirits (probably).		Not objectionable	Old fatty oil smell	do	Objectionable	Do.
Objectionable to work with.	1. Gum spirits		Not objectionable	Not objectionable	None seriously objectionable.	This flows heavily	Can work with all. No. 3 most obnoxious and can not work any length of time in closed room.
	2. Steam distilled spirits.		do.				
	3. Destructively distilled spirits.		Objectionable				
	4. Destructively distilled spirits.		do.				
	5. Gum spirits (probably).		Not objectionable				
Are difficult to work with.	1. Gum spirits	None are	None are	None are	Not difficult	None are	All work nicely.
	2. Steam distilled spirits.				Difficult		
	3. Destructively distilled spirits.				do.		
	4. Destructively distilled spirits.				do.		
	5. Gum spirits (probably).				Not difficult		

Varnishes rub down.	1. Gum spirits	....	None are satisfactory from a polisher's standpoint.	All too slow and too elastic to rub; can not be rubbed.	Rubs out well, good surface.	Medium in rubbing.	All rub well.
	2. Steam distilled spirits.				Rubs fairly well, fair surface.		
	3. Destructively distilled spirits.				Rubs good quickly, fair surface.		
	4. Destructively distilled spirits.				Rubs fair quickly, fair surface.		
	5. Gum spirits (probably).				Rubs well, good surface.		
Behavior under the brush.	1. Gum spirits	....	Flows freely	All flow too freely for the class of varnishes.	Works rather stiff	This flows heavily	
	2. Steam distilled spirits.				Works very free	....	
	3. Destructively distilled spirits.				do	....	
	4. Destructively distilled spirits.				Works fair	....	
	5. Gum spirits (probably).				Works free	....	
General remarks	....	All unsatisfactory; did not flow out well. The first was best, third, second best, and the other three equally poor.	After 15 days drying all the samples were polished with great difficulty.	....	The spreading, drying, and rubbing qualities are from fair to good. The pure turpentine varnishes giving best results.	We consider all of them very medium varnishes.	

PIANO RUBBING VARNISHES.

Varnishes were thinned with—	1. Gum spirits	Not thinned	1 part thinner to 4 parts varnish.	1 part thinner to 4 parts varnish.	2 parts thinner to 1 part varnish.	All thinned alike 1st coat 1 to 1 2d coat 1 to 1.4 3d coat 1 to 1.4 4th coat 1 to 5 5th coat 1 to 5 6th coat 1 to 5	All thinned alike (120 cc varnish to 30 cc turpentine).
	2. Steam distilled spirits.		1 part thinner to 5 parts varnish.	1 part thinner to 7 parts varnish.	Not thinned		
	3. Destructively distilled spirits		do	1 part thinner to 21 parts varnish.	do		
	4. Destructively distilled spirits		do	Not thinned	do		
	5. Gum spirits (probably).		do	1 part thinner to 21 parts varnish.	do		
Varnishes remained tacky.	1. Gum spirits	9 hours	3½ hours	6 to 7 hours	About 36 hours	About 3 hours	8 hours.
	2. Steam distilled spirits.	5½ hours		7 to 8 hours		do	6½ hours.
	3. Destructively distilled spirits.	4½ hours		6 to 7 hours		About 2 hours	8 hours.
	4. Destructively distilled spirits.	7 hours		do		do	8½ hours.
	5. Gum spirits (probably).	5½ hours		do		do	7½ hours.

TABLE 11.—*Detailed opinions of the workmen on the varnishe used*—Continued.

PIANO RUBBING VARNISHES—Continued.

Data.	Varnish.	A. B. Chase Co.	Wm. Knabe & Co.	Murphy Varnish Co.	Devoe & Raynolds Co.	Steinway & Co.	American Paint Manufacturing Association.
Varnishes hardened in—	1. Gum spirits	Allowed one week between coats on all.	Appeared hard in 5 days. Allowed 10 days.	18 hours	48 hours	8 to 12 days	40 hours.
	2. Steam distilled spirits.			22 hours	72 hours		36 hours.
	3. Destructively distilled spirits.			20 hours	do		Do.
	4. Destructively distilled spirits.			18 hours	do		40 hours.
	5. Gum spirits (probably).			20 hours	48 hours		36 hours.
Varnish was applied in—	1. Gum spirits	3 coats	3 coats	3 coats, 3 days between coats.	4 coats	6 coats	4 coats.
	2. Steam distilled spirits.						
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.						
	5. Gum spirits (probably).						
Gloss of varnish surface.	1. Gum spirits	No perceptible difference in gloss.	Can not distinguish any difference.	Luster fair		Medium gloss	All good. No. 2 best, Nos. 1 and 3 show tears.
	2. Steam distilled spirits.			Luster full and rich, best.		do.	
	3. Destructively distilled spirits.			Good and full, second best.		do.	
	4. Destructively distilled spirits.			Luster fair		Fair gloss	
	5. Gum spirits (probably).			Good and full, third best.		do.	
Odor is objectionable.	1. Gum spirits			Smells of naphtha			Objectionable.
	2. Steam distilled spirits.	Objectionable		Smells of York spirits.	Strong resinous odor.	Objectionable	
	3. Destructively distilled spirits.			do.	do.	do.	
	4. Destructively distilled spirits.	Objectionable	Objectionable	Worst of any	do.		
	5. Gum spirits (probably).			O. K.			
Objectionable to work with.	1. Gum spirits	Samples not large enough to say definitely.	Not objectionable			Not objectionable	All work nicely. Can work with all. No. 3 most objectionable and can not work any length of time in closed room with No. 4.
	2. Steam distilled spirits.		do.				
	3. Destructively distilled spirits.		do.	Sets too quickly, pulls.			
	4. Destructively distilled spirits.		On account of odor.	Objectionable			
	5. Gum spirits (probably).		Not objectionable				

Are difficult to work with.	1. Gum spirits		Not difficult.		Difficult	Not difficult.	
	2. Steam distilled spirits.				do		
	3. Destructively distilled spirits.			On large surface	do		
	4. Destructively distilled spirits.				do		
	5. Gum spirits (probably).						
The varnishes rub down.	1. Gum spirits	Satisfactorily	Satisfactorily	All rub well but sweat soon after. No. 3 is toughest.	Rubs out well, good surface.	Satisfactory	All rub well.
	2. Steam distilled spirits.				Rubs out fair, fair surface.		
	3. Destructively distilled spirits.				Rubs out quickly, fair surface.		
	4. Destructively distilled spirits.				do		
	5. Gum spirits (probably).				Rubs out well, good surface.		
Behavior under brush.	1. Gum spirits		Flows well	All good on the small surfaces used.	Works a little stiff	All flow freely from brush.	No difficulty in flowing with any.
	2. Steam distilled spirits.	Does not flow freely.			Very free		
	3. Destructively distilled spirits.	do			do		
	4. Destructively distilled spirits.	do			Fair working		
	5. Gum spirits (probably).				Works free		
Character of body	1. Gum spirits	Good body	Heaviest body				
	2. Steam distilled spirits.	do					
	3. Destructively distilled spirits.	do					
	4. Destructively distilled spirits.	Body not so good					
	5. Gum spirits (probably).	do					
General remarks		These samples rubbed better than the regular varnish that we use.	Time and exposure can only determine accurately the value of the varnishes.		We find it difficult to form an opinion as to the merits of these goods. The best working results can not be obtained with Nos. 2, 3, and 4.		

TABLE 11.—*Detailed opinions of the workmen on the varnishes used*—Continued.

COACH FINISHING VARNISHES.

Data.	Varnish.	Devoe & Raynolds Co.	Murphy Varnish Co	H. H. Babcock Co.	Fay & Bowen Engine Co.	James & Meyer Buggy Co.[1]	Paint Manufacturing Association of the United States.
Varnishes were thinned with—	1. Gum spirits	1 part thinner to 8 parts varnish.	1 part thinner to 3 parts varnish.	1 part thinner to 12 parts varnish.	1 part thinner to 8 parts varnish.	1 part thinner to 20 parts varnish.	All thinned alike 120 cc. varnish thinned with 30 cc. of the proper turpentine.
	2. Steam distilled spirits.	Not thinned	Not thinned		Not thinned		
	3. Destructively distilled spirits.	do	do		do		
	4. Destructively distilled spirits.	do	do		do		
	5. Gum spirits (probably).	do	1 part thinner to 13 parts varnish.		do		
Varnishes remained tacky.	1. Gum spirits	Average 36 hours	4 hours	About 5 hours	About 3 hours	7 hours	3 hours 5 minutes.
	2. Steam distilled spirits.		5 hours	About 6 hours	About 4 hours	4½ hours	2 hours 40 minutes.
	3. Destructively dis-spirits.		4 hours	do	do	4 hours	Do.
	4. Destructively distilled spirits.		6 hours	do	do	3¾ hours	2 hours 20 minutes.
	5. Gum spirits (probably).		4 hours	do	About 3 hours		2 hours 15 minutes.
Varnishes harden in—	1. Gum spirits	Average 48 hours	Less than 12 hours	About 24 hours	8 to 10 hours	36 hours	54 hours.
	2. Steam distilled spirits.				10 to 12 hours	do	47 hours.
	3. Destructively distilled spirits.				do	30 hours	51 hours.
	4. Destructively distilled spirits.				do	24 hours	49 hours.
	5. Gum spirits (probably).				8 to 10 hours		Do.
Varnish was applied in—	1. Gum spirits	2 coats	1 coat	1 coat	3 coats	2 coats	4 after filler.
	2. Steam distilled spirits.						
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.						
	5. Gum spirits (probably).						

Gloss of varnished surface.	1. Gum spirits		Very little difference. All look more like piano varnishes than coach varnishes.		Good	No difference in gloss.	All good. Nos. 3 and 5 best. Nos. 1 and 3 show tears.
	2. Steam distilled spirits.				Poor first coat; second and third better.		
	3. Destructively distilled spirits.				do		
	4. Destructively distilled spirits.			Best gloss and general appearance.	do		
	5. Gum spirits (probably).				Good		
Odor is objectionable.	1. Gum spirits					Objectionable	
	2 Steam distilled spirits.	Objectionable		Objectionable	Objectionable		
	3 Destructively distilled spirits.	do			do		Objectionable.
	4. Destructively distilled spirits.	do	This is worst	Objectionable	do		Do.
	5. Gum spirits (probably).		Only one that smells of straight turpentine.				
Objectionable to work with.	1. Gum spirits	None seriously	On account of odor only.			None objectionable.	Can work with all, but No. 3 is most obnoxious. No. 4 can not be worked any length of time in closed room.
	2. Steam distilled spirits.			This is; odor	In odor and tight body.		
	3. Destructively distilled spirits.				do		
	4. Destructively distilled spirits.			This is; odor	do		
	5. Gum spirits (probably).						
Are difficult to work with.	1. Gum spirits	This is	None are	None are	None are	None are	They all work nicely.
	2. Steam distilled spirits.						
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.	This is					
	5. Gum spirits (probably).						
The varnishes rub down.	1. Gum spirits					Satisfactory	All rub well.
	2. Steam distilled spirits.		Not rubbed				
	3. Destructively distilled spirits.						
	4. Destructively distilled spirits.						
	5. Gum spirits (probably).						

[1] The regular varnish used in striping buggy gears was thinned with the four turpentines.

TABLE 11.—*Detailed opinions of the workmen on the varnishes used*—Continued.

COACH FINISHING VARNISHES—Continued.

Data.	Varnish.	Devoe & Raynolds Co.	Murphy Varnish Co.	H. H. Babcock Co.	Fay & Bowen Engine Co.	James & Meyer Buggy Co.	Paint Manufacturing Association of the United States.
Behavior under the brush.	1. Gum spirits	Not free; stiff	Works full and well.	Flows fairly well	Does not flow very freely.	Best flowing	No difficulty in flowing.
	2. Steam distilled spirits.	Flows fair	do.		Flows very freely		
	3. Destructively distilled spirits.	Flows free	Works too full		do.		
	4. Destructively distilled spirits.	do.	Works too thin		Not as free as Nos. 2 and 3.		
	5. Gum spirits (probably).	Flows very free	Works full and well.		do.		
Character of body	1. Gum spirits	Heavy body			Little heavy		
	2. Steam distilled spirits.	Medium body			Very light body		
	3. Destructively distilled spirits.	do.			do.		
	4. Destructively distilled spirits.	do.			do.		
	5. Gum spirits (probably).	do.					
General remarks	1. Gum spirits			Silky appearance	Think that the first and the last are the best varnishes.	Works like regular turpentine.	
	2. Steam distilled spirits.			do.		Cracks and crawls apart in striping.	
	3. Destructively distilled spirits.			Silky appearance; loses luster.		Works like regular turpentine.	
	4. Destructively distilled spirits.			Best of lot		Cracks and crawls apart in striping.	
	5. Gum spirits (probably).			Silky appearance			

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BY

WILLIAM B. ALWOOD,
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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY—BULLETIN No. 145.
H. W. WILEY, Chief of Bureau.

ENOLOGICAL STUDIES.

THE CHEMICAL COMPOSITION OF AMERICAN GRAPES GROWN IN OHIO, NEW YORK, AND VIRGINIA.

BY

WILLIAM B. ALWOOD,
Enological Chemist.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., March 27, 1911.

Sir: I have the honor to submit for your approval a report on the results of a three-year investigation of the composition of American grapes, conducted at Charlottesville, Va., and Sandusky, Ohio, by William B. Alwood, the enological chemist of this bureau, covering the principal commercial varieties of American grapes grown in the central and eastern sections of the country, and also other varieties not generally cultivated. These data, even though the study is far from complete, are of practical importance to the grape growers and manufacturers of grape by-products, as well as of scientific interest, and I recommend their publication as Bulletin No. 145 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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PREFACE.

In the conduct of certain enological work it became evident that there were almost no chemical data available regarding the character of grapes grown in Virginia, in northern Ohio, in northern and central New York, and in some other parts of the country. The condition was much the same as that which was found in the beginning of the enological studies on the production of cider. It is evident that in order to treat a subject of this kind in a rational way it is important that the character of the fruits under consideration should be definitely known. After the investigation on the composition of the grapes in the Piedmont region of Virginia was begun it was found advisable to establish a laboratory, or laboratories, for further studies on the composition of this fruit and the products made therefrom in the localities which have become so famous for the production of grapes, grape juices, and wines. The establishment of a branch of the enological investigations at Sandusky, Ohio, rendered it possible to study in situ the character of the grapes produced in that region. This study has now continued over a period of three years, and data are at hand for drawing at least preliminary conclusions respecting the general character of the fruit produced. As is the case in every grape-growing country, it has been found in northern Ohio and central and northern New York that the character of the grape varies from season to season, and often in the same vineyard or locality during a given season. This variation shows the necessity which exists in this locality, as well as everywhere else, of securing by careful selection the grapes which are to be used for specific purposes. Those that are sound, healthy, mature, and suitable for the purpose should be separated from the imperfect, unripe, damaged, or otherwise undesirable varieties. In the manufacture of unfermented grape juice, for instance, it is desirable that the grapes yield a juice which is palatable, wholesome, and easily treated. It is not desirable to bottle the fresh juices and sterilize them without removing, in so far as possible, the sedimentary matters which will be deposited on heating and standing. Many varieties of grapes lend themselves much more readily to treatment of this kind than others. The chemical composition and physical properties of the fruit and its amenability or resistance to treatment, either in the preservation of the juice in an unfermented state or in the manufacture of wine, are of the greatest importance. As is well known, the predominant acid in ripe grapes is tartaric. The presence of this acid, either in a free state or in combination with lime or potash, is necessary to give to the grapes the peculiar flavor for which they are noted. In other words, the acid content of the grape is one of the ingredients of chief importance from an organoleptic point of view. A grape which is without acid would be totally insipid, and would yield a juice not much better than sweetened water. On the other hand, a grape juice in which the acid is excessive is unpalatable, at least to most persons. Hence the production of a grape with the proper degree of acidity to give character and not enough to impair taste is of great importance.

The studies which have been made and which are detailed in the following pages throw much light on this important problem, even though they are far from complete, indicating what varieties of grapes and what conditions of environment give the best results.

Equally important from the point of view of vinification is the sugar content of the grape. The only source of natural alcohol in wine is fermentation of the sugars which exist in the original grape. These sugars exist in combination with other bodies, many of which enter into the finished product and serve to give it character and quality. In fact, the smaller the quantity of alcohol which can be secured in the finished wine, and at the same time permit of its manufacture and keeping, the better. Other things being equal, the wine which has the smallest percentage of alcohol is to be preferred. The data which have been secured indicate the varieties of grapes which give the best percentage of sugar for proper fermentative purposes.

These studies will lay broad and deep the foundation for the practical working out of the problem of the utilization of grapes for the various purposes for which grape products are employed. While the work has been carried on under the general direction and supervision of the Chief of the Bureau of Chemistry, the details of it and the technique of the processes have been in the hands of Mr. William B. Alwood, who has given to the work his undivided attention and has with enthusiasm and success attacked the many problems which have been presented.

H. W. Wiley,
Chief of Bureau.

Washington, D. C., *March 10, 1911.*

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ENOLOGICAL STUDIES.

THE CHEMICAL COMPOSITION OF AMERICAN GRAPES GROWN IN OHIO, NEW YORK, AND VIRGINIA.

OBJECT OF THE INVESTIGATION.

In the discharge of duties devolving upon the Bureau of Chemistry under the food and drugs act it became apparent that definite data on the methods of wine making and of the products made in the several wine districts of the country were needed. As chemical data on the composition of the fruit used in the manufacture of grape juices and wines were not available, it was found necessary to make a fairly complete fundamental study of the grape and the by-products made from this fruit. As a first step in this direction a detailed study was made of the chemical composition and general character of the several varieties of grapes grown in the Central and Eastern States, especially those sent to the wineries at Sandusky, Ohio, with a view to determining the suitability of these grapes for food purposes and for wine production. Further than this a chemical study of the commercial wines produced in the middle and eastern wine-growing districts was also found necessary for the purpose of comparing their quality with that of wines which might be made under the direction of the bureau from the grapes commonly used at the wineries for this purpose.

The need of this investigation in the administration of the food law is readily seen if one compares the widely varying statements of the grape growers and the wine makers as to the quality of the fruit produced and the possibility of making straight wines from this fruit, and also if one is familiar with the general practice of watering and sugaring (i. e., gallizing) the wines made in the districts mentioned. For these reasons it is important to determine fully the character of the strictly nature wines made from these grapes. There appeared to be no other method of securing these data than by a thorough chemical investigation covering the whole subject of the fruit grown and the character and composition of the wines produced and sold in the districts in question. The term "American grapes" is here used to distinguish native seedlings and crosses of these with the

European species produced in America, from the distinctly European varieties grown so largely in California.

It is claimed by some that the grapes of New York State and the Atlantic seaboard States are richer in sugar and not so acid as the fruit grown in the Lake Erie district. Because of the proximity to a very large grape-growing industry and of the important wine industry located there the work has thus far been carried on chiefly at Sandusky, Ohio, but it is intended to extend it as rapidly as possible to other important centers. A large portion of the northern grape belt has already been covered in this preliminary investigation, and the importance of the crop and its by-products and the necessity for accurate information seem to fully justify this course. But the data now in hand will render possible more detailed investigations of certain specific features—as, for instance, the variability in composition of the more important varieties under differing conditions of soil, climate, and culture—which appear to promise results of much practical importance.

GRAPES GROWN IN OHIO AND NEW YORK.

METHODS OF OBTAINING SAMPLES.

In an investigation of this nature the character of the sample used is of great importance. The data for the individual samples show that the difference in composition between samples from the same source may be sufficient to be confusing. Hence, with a view to forestalling hasty conclusions, a very large number of samples, from as many sources as possible, have been examined. This should render the averages presented quite reliable. There will certainly be a very considerable variation in composition from year to year, the extent of which can only be determined by continuing these examinations until sufficient data have been secured to furnish a basis for a final statement.

The season was so far advanced when this work was begun in 1908 that arrangements could not be made to secure samples from a large number of individual growers; hence they were taken at the wine cellars. But for the years 1909 and 1910 a large number of samples were secured directly from the growers, and to check against the results so obtained samples were taken from the wine cellars. In getting samples from growers the selection of "extra quality" has been prevented as far as possible, the aim being to use the average commercial fruit. In any case the large number of samples examined from the various sources minimizes the incidental variations so as to warrant a reasonable belief that the averages herein presented represent the quality of the fruit for the season in question.

The two elements of prime importance in the grapes used for the manufacture of unfermented grape juice, for wine making, or for other by-products, are the amount of sugar and the amount of acid present in the fruit; hence the work undertaken in 1908, the first season at Sandusky, was confined almost wholly to sampling and examining crops of grapes found at the various wineries and juice factories, and to observations of the methods of vinification employed. A small amount of wine was made for technical study. It was unfortunate that the investigations could not have been begun at the opening of the vintage season in 1908, because the crop was especially good that year for making high-class products, but it was impossible to make preparations for more extensive operations than those indicated.

PREPARATION OF THE SAMPLE FOR ANALYSIS.

The samples of fruit secured for 1908 were taken at the various wine cellars directly from the packages as they arrived at the cellars, and the name and address of the grower was invariably entered on the records with each sample. About 4 pounds of fruit were taken for a sample, and care was taken to secure an average of the crop. These samples were crushed and pressed by hand in the laboratory of the Duroy & Haines Co., who kindly furnished facilities for this work.

The preparation of the samples is a matter of considerable importance. Experiments on crushing in a mortar by using a pestle, on crushing by hand in porcelain vessels, and also with small hand grinders were made. After careful tests by these methods it was found that crushing by hand is the best method of procuring a sample. In this way each grape is crushed practically to the same extent, and the whole mass is reduced to a pulp so much more homogeneous than by any other method tried, that this one was adopted for all work during the years 1909 and 1910. The stems were in every case left in the pulp.

When crushed to a pulp the sample was transferred to a piece of cheesecloth folded double, spread over a porcelain dish, and the pulp was pressed by gathering the edges of the cloth firmly in one hand and gently squeezing the mass until the juice was extracted. The pulp is in this manner reduced to a ball in the cloth, and by gripping this firmly with the free hand and applying pressure properly a careful operator can reduce the pulp to about the condition of dryness arrived at by applying 1,500 pounds direct pressure in a hydraulic press. The amount of pressure used will affect considerably the acid present in the expressed juice; hence care is required in this manipulation to secure comparable results.

TESTS AND ANALYSES MADE.

Each sample of juice was then tested as to density with a Brix spindle, and in most instances a sample was saved for further examination. These samples for the year 1908 were bottled and preserved with mercuric chlorid, using tablets purchased at the drug store, and in this condition they were sent to Stonehenge laboratory, Charlottesville, Va., for analysis. The final analyses were completed by January 5, 1909, and covered only specific gravity, total solids, sugar-free solids, total sugar, and fixed, volatile, and total acid. These data were carefully checked, and in the acid determinations allowance was made for the use of the mercuric chlorid tablets, each of which contained 0.5 gram of mercuric chlorid and 0.57 gram of ammonium chlorid. One tablet was used for 400 cc of fresh juice. A correction to allow for the acid present in the tablet was made by deducting 0.075 gram tartaric acid per 100 cc of sample. Volatile acid was determined on the samples taken in 1908, but the amount was so slight that it is not reported in the tables; seldom was 0.01 gram per 100 cc of sample found. During the years 1909 and 1910 volatile acid determinations were not made.

The results on total acid for the samples taken in 1908 are surprisingly low. This was noted as the samples were analyzed, and the work was carefully checked. The only explanation which is suggested is that during the first season the samples were not pressed sufficiently to bring out all the acid present. It will be readily understood that for red wines the acid content secured by crushing and pressing the fresh fruit will not be the total acid secured by the fermentation of the pulp and then pressing by power, as is customary in the wineries, but for white wine the result should be practically the same as when the fruit is crushed and pressed by power. These points will be covered and the data given in an additional report on the fermentation experiments conducted at the Sandusky laboratory.

The preparation of the samples during the years 1909 and 1910 was uniformly by hand as just described; but, having equipped a field laboratory at Sandusky, the analyses of the juice were made there at once, as far as possible. In every case the acid was determined by the titration of the freshly pressed and filtered juice, and a gravity reading was made on unfiltered juice at the same time, using a Brix spindle specially made for this work. When the analysis could not be made promptly a sample was preserved in a pint champagne bottle with 0.2 gram of mercuric chlorid. The full analysis was completed usually within from 30 to 60 days after sampling. If the presence of sucrose was suspected the analysis was made at once, because experience showed that inversion of this form of sugar occurred even in the chemically preserved sample.

The methods of analysis followed are those laid down in Bulletin No. 107, Revised, Bureau of Chemistry; certain details for which no explicit directions are given were made to conform to well settled laboratory practice. Total solids were derived as for wine from the table given in Bulletin No. 107, Revised (p. 218 et seq.), and the sugar-free solids by subtracting the total reducing sugar from the total solids, save in cases where the sample showed sucrose. In such instances the sugar-free solids were obtained by subtracting the sum of the reducing sugar and sucrose from the total solids. Subtracting the total sugar found by inversion when sucrose is present results in reducing the sugar-free solids below normal.

The acid determination was made on filtered juice without previous heating, by titrating with tenth-normal sodium hydroxid, free from carbonate, using a neutral solution of azolitmin on a spot plate as indicator. When the juice was highly colored it was found advantageous to dilute the portion taken about two and one-half times with recently boiled but cold water, that the reaction might be more clearly seen.

The sugar determinations were made after the methods of Munson and Walker (Bulletin No. 107, Revised, p. 241), but it was found possible to use the gooches twice without cleaning. This was definitely determined by trial, as shown by the following check results:

Reducing sugar determinations to test need of cleansing gooch before each determination.

[Grams per 100 cc.]

Filtered and weighed on clean pad:		Filtered and weighed on 4.3 decigrams cuprous oxid:	
White grape juice—		White grape juice—	
1	21.11	5	21.01
2	21.13	6	21.05
3	21.11	7	21.08
4	21.11	8	21.08
Average	21.12	Average	21.06
Red grape juice—		Red grape juice—	
1	16.88	5	16.83
2	16.87	6	16.91
3	16.85	7	16.85
4	16.91	8	16.83
Average	16.88	Average	16.86

The Brix readings given are those made on the fresh juice, with a standardized Brix must spindle especially devised by this laboratory and corrected to 20° C. in accordance with the regulations of the Bureau of Standards. The specific gravity readings given were in every case determined by pyknometer when the sample was analyzed. These notes apply to the work of 1909 and 1910; some slight variations from this detail occurred during the preliminary work of 1908.

INVESTIGATION OF 1908.

The data for each variety sampled in 1908 are summarized in Table I, page 20, by bringing the results on all the samples of a variety from each locality together and giving the average; if more than one sample was analyzed, the maximum and minimum results for each locality are shown. The figures presented in this table cover too few samples to warrant any extended discussion; from several important localities only one sample of a given variety was examined. This was due to lack of time to organize the work completely for that year. Further, the data are deficient in that the territory was not adequately covered. These criticisms, however, do not deprive these data of value, and, as far as they go, the results show a remarkably high sugar content in the great majority of the samples analyzed. In fact, the grape crop was of very good quality that year, and it was often stated that not in 20 years could such quality be expected again. Only one sample of Catawba was analyzed from Middle Bass and one from North Bass, Ohio, but the Brix readings on the other samples from these localities show that they were all richer in solid matter and hence presumably were higher in sugar than the two analyzed. The importance of the Brix readings of fresh must, as a means by which the grower can determine with considerable accuracy the sugar content of the fruit, is worthy of special attention.

The data[1] show that 19 samples of Ives, notably the poorest grape in sugar content of those used for wine in the Sandusky district, gave an average percentage composition of 15.06 per cent of sugar and 0.485 of total acid. Concord, which is usually not much superior to Ives, shows for 11 samples 16.20 per cent of sugar and 0.434 of total acid. Clinton, a variety not largely grown, showed in 5 samples 17.76 per cent of sugar and 0.998 of total acid. Norton, a grape apparently not well adapted to the Lake district, gave for 3 samples an average of 22.79 per cent of sugar and 0.907 of total acid. The fermented pulp will, of course, give a wine that is still more acid.

It is especially important, however, to note that Catawba, the chief white wine grape of the Lake district, on an average of 25 samples, showed 18.81 per cent of sugar and 0.750 of total acid. Delaware, which is well known for its high quality, showed for 2 samples an average of 22.84 per cent of sugar and 0.423 of total acid; and Iona, a superb wine grape, but not extensively grown, in one sample had 19.35 per cent of sugar and 0.623 of total acid. These results as to sugar content indicate a very high quality of fruit.

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[1] For percentage data see table on page 16.

INVESTIGATION OF 1909.

During the season of 1909 preparation was made to extend the work by equipping a laboratory at Sandusky, Ohio. Accommodation was thus provided for handling a large number of samples and for making fermentation experiments on the different varieties of wine grapes. The report on the fermentation experiments is not, however, included in this report, which deals only with the composition of the fruit samples. A canvass of the Lake Erie district and as far east as the central New York grape district was made during August and September, 1909, and correspondents were selected at several points of importance who were relied upon for samples of the crop for the laboratory work, and a number of whom rendered valuable assistance. The samples were sent by express or boat, usually in small baskets, to the Sandusky laboratory, where the notes on quality and the chemical examinations were made.

This arrangement furnished a large number of samples direct from the growers, but others were also secured from the wine cellars in Sandusky at such periods as seemed most likely to furnish data on the full range of the fruit crushed for wine. However, samples from the wine cellars were not usually considered as representing individual crops during 1909, but were regarded as a composite of the stock on hand at the time the sample was taken. From the American Wine Company composite samples were taken of the expressed juice as it was collected in the settling tank. Such samples are reported under the names of the several cellars.

The data determined at the time of sampling during 1909 comprise a Brix reading on the fresh must, and titration for total acid as soon as the sample was crushed and pressed; in most cases the sample was further analyzed by determining the specific gravity by pyknometer, the total solids, sugar-free solids, and total sugar. Inversion was only resorted to when the results showed excessive sugar-free solids by direct determination. Volatile acid was not determined on the fresh fruit during 1909, as the data for the previous year showed this to be unimportant. Samples which were not analyzed as fresh fruit were treated with pure mercuric chlorid and stored for analysis later, 0.2 gram being used for 400 cc of juice. A correction for the use of mercuric chlorid was found to be unnecessary when the pure crystallized salt is used. The results on acid in a juice are usually lower if the sample has stood in a bottle for some time, owing to the precipitation of crude "tartar." Tests on a number of varieties showed this to amount approximately to 0.064 gram per 100 cc for white juice and 0.051 gram for red juice. The results given in the table are not, however, affected by this fact, because the acid was determined in the fresh juice.

The results for 1909 are summarized by varieties, bringing together all the samples collected in each locality. These data are found in Table II (p. 21). The composite samples from the individual wine cellars are arranged together without reference to the locality from which the crops came.

The year 1909 was remarkable for the very heavy crop of fruit borne on the vines. It was commonly remarked by the growers that such an abundant crop had seldom been produced. But unfortunately the latter part of the season was very unpropitious for the ripening of the load of fruit with which the Catawba vines were weighed down. Cold, rainy weather set in when the main crop was barely colored, and this continued with slight intermission until actual freezes injured the foliage, with the result that in some vineyards a considerable portion of the crop did not mature and was either a total loss or was sold at low prices—from $12 to $15 per ton. A considerable number of these pale Catawba samples were tested for sugar and acid and found to run from 2 to 4 per cent lower in sugar and about 2 per mille, i. e., per 1,000, or 0.2 per cent, higher in acid than the average mature grapes. In 1909 every variety to which special attention has been called (namely, Clinton, Concord, Ives, and Norton of the red-juice grapes, and Catawba, Delaware, and Iona of the white-juice grapes), shows a sugar content from 1 to 4 per cent lower than in 1908, and the acid is higher. Yet even in this admittedly very unfavorable year the grapes were, on the whole, of a good quality. It is notable that out of 16 samples of Catawba juice secured from a single cellar only 2 fell below the average of 16.31 per cent of sugar for the entire 84 samples from all sources in Ohio, and that the acid was only slightly higher than the average in 8 instances out of the 16. Thus even in a bad crop year it is shown that fruit of very fair quality can be selected. The 16 juice samples mentioned were from selected crops bought for making champagne wine and indicate that the quality of the fruit can be greatly affected by the method of growing the crop.

INVESTIGATION OF 1910.

The season of 1910 was marked by the almost total failure of the grape crop in some sections of the northern fruit belt, due to the severe late spring frosts. However, the Lake Erie and central New York districts gave about 40 to 60 per cent of a crop. On the whole the quality of the crop was in most instances quite equal to that of 1908. The sampling work was extended to cover more fully the two districts mentioned, and a few samples of Concord grapes were secured from Michigan. The manipulation of the samples was the same as previously noted, and all deferred analyses were completed by December 20, 1910. A large number of varieties were analyzed which, judging from the results, are of little importance for the manufacture of grape products, but it was thought best to include in the investigation all varieties found in the districts covered for a period of several years, so that the data accumulated might be exhaustive.

During the season of 1910 the work was furthered by the hearty cooperation of the New York Agricultural Experiment Station, both at the main station at Geneva and at the grape station at Fredonia, N. Y. Prof. Hedrick and his colleagues collected and forwarded all the samples from Geneva and from the Chautauqua grape belt. In the Lake Keuka district the very efficient cooperation of Mr. L. D. Masson at Hammondsport, N. Y., and of Mr. W. N. Wise at Penn Yan, N. Y., was secured. For the important grape district about North East, Pa., Mr. Fred Johnson, agent and expert for the Bureau of Entomology, in charge of the investigation of grape insects at that point, was authorized to collect and forward samples of fruit to the Sandusky laboratory, which he did in a most satisfactory manner. For the Lake Erie islands Mr. Henry Elfers at Kelleys Island, Mr. H. J. Burns at Middle Bass, and Mr. R. Siefield at North Bass, rendered very valuable assistance in collecting and forwarding samples.

The tabular presentation of the analyses for 1910 follows the same plan as that of the two previous years and is sufficiently comprehensive to warrant careful study both by the grape grower and the manufacturer of grape by-products. (See Table III, p. 27.) It is not warrantable at this time to theorize on the results of these three years of investigation further than to point out the remarkably good quality of such varieties as Catawba, Delaware, Iona, and others of the grapes with light-colored juice and also the rather unexpected high sugar content shown by Concord. Norton and Clinton have a very good percentage of sugar, but such a very high percentage of acid as to render their value for the northern grape belt problematical. But Montefiore, which is so little grown that it could not be studied sufficiently to determine its value with any certainty, has much promise because of the low acid and the high sugar content. If it can be successfully cultivated this should prove an important grape.

A tabular statement of the average sugar and acid content for four of the red and three of the white juice grapes commercially accepted as the best for wine making in the northern grape belt is given in the following table, which covers the data for the three years during which the work has been in progress. During the first year only Ohio samples were examined, but after that time samples from other localities were included, except in the case of Norton. The Ohio samples are given separately and then the data for all of the samples together, since that district is so much better represented in most instances, and also, thus far, presents some indications of a higher sugar and lower acid content than fruit from the other States. This is an interesting point, but it must be confirmed by a more complete examination of fruit from the other sources before a definite statement can be made.

The varieties compared include, as stated, some of the most important commercial varieties, and also represent the three groups of native grapes from which our cultivated varieties are mostly derived, namely, the Riparia, Aestivalis, and Labrusca groups, as well as Vinifera hybrids. Further investigations, dealing more critically with a large number of varieties of each of these groups, are much needed.

The Delaware and Iona (Labrusca and Vinifera hybrids) have a remarkably high sugar and low acid content; Catawba, a Labrusca, with possibly a strain of Vinifera parentage, shows fairly high sugar and also high acid; the Ives and Concord, pure natives of the Labrusca group, are low in acid and not sufficiently high in sugar to make a claret wine of the usual strength; that is, from 9 to 10 per cent of alcohol. Clinton represents the Riparia and Norton the Aestivalis group of our native species. Both of these can be grown with sufficient sugar content to make a fine, sound wine, but they are strongly acid.

Comparison of average sugar and acid content of important varieties for three years.

[Percentage data calculated from results expressed in grams per 100 cc (Tables I-III) by dividing by the specific gravity.]

Variety and year.	Samples from Ohio.				Samples from all sources.			
	Total sugar.		Total acid.		Total sugar.		Total acid.	
	Number of samples.	Amount.	Number of samples.	Amount.	Number of samples.	Amount.	Number of samples.	Amount.
Clinton:		*Per cent.*		*Per cent.*		*Per cent.*		*Per cent.*
1908	5	17.76	5	0.998	....	....	....	....
1909	12	16.14	12	1.553	16	16.39	16	1.638
1910	3	18.99	3	1.755	7	18.12	7	1.872
Concord:								
1908	11	16.20	11	.434	....	....	....	....
1909	46	14.92	40	.646	76	14.80	69	.681
1910	30	16.77	30	.693	88	15.75	88	.785
Ives:								
1908	19	15.06	19	.485	....	....	....	....
1909	27	14.05	26	.721	30	13.76	29	.720
1910	18	14.79	18	.693	22	14.82	22	.720
Norton:								
1908	3	22.79	3	.907	....	....	....	....
1909	11	17.84	11	1.657	....	....	....	....
1910	5	19.53	5	1.534	....	....	....	....
Catawba:								
1908	25	18.81	25	.750	....	....	....	....
1909	84	16.31	84	1.067	91	16.59	91	1.080
1910	84	19.20	84	.984	93	19.01	93	1.016
Delaware:								
1908	2	22.84	2	.423	....	....	....	....
1909	22	18.96	17	.748	36	18.92	31	.762
1910	11	22.02	11	.720	28	19.57	28	.803
Iona:								
1908	1	19.35	1	.623	....	....	....	....
1909	3	17.82	3	.757	4	18.18	4	.770
1910	5	19.51	5	.793	8	19.16	8	.921

SEEDLINGS AND UNKNOWN VARIETIES.

In covering as large a territory as that from which the samples listed in this report have been collected, naturally a number of seedlings or unknown varieties have been received. Of these, nine appear to merit special attention. For convenience only, these are numbered from 1 to 9.

The first mentioned in the table which follows was found in the vineyard of Mr. N. Schneider at Vermilion, Ohio. He states that it is a seedling grown by him some years ago direct from Catawba, and the characteristics of the vine and the fruit bear out his statement as to parentage. It has a more neutral flavor than Catawba and when fully ripe tastes less acid, but the analyses do not show much in its favor as compared with the parent stock. However, its vinous character deserves attention.

The second of these seedlings was found in the vineyard of Mr. E. L. Steuk, at Venice, Ohio, and is of unknown parentage. The full discussion of this grape given in Bureau of Chemistry Bulletin 140 renders further consideration unnecessary. Yet it is important to call attention to its very low acid and fairly high sugar content. The flavor is very mild, almost insipid when ripe. The fruit is somewhat of the Iona type.

The seedling secured from Mr. J. H. Bauman, Kelleys Island, Ohio, is high in sugar and has a very desirable type of bunch, the fruit is red in color, with white juice and good flavor. No sample of this fruit was secured in 1910.

The sample from Mr. L. C. Dodge, Middle Bass Island, Ohio, did not show up well in the analysis for 1909, but in 1910 it had a high sugar content, low acid, and mild flavor. The fruit is like Catawba in type, and it is perhaps a seedling of this variety.

The seedling from Mr. N. Wagner, Brownhelm, Ohio, showed remarkably high sugar and low acid for the year 1909. No sample was secured in 1910.

The sample from Mr. H. Kümmel, Middle Bass Island, Ohio, shows only a fair amount of sugar, but the acid is low and the flavor very good, being mild and vinous. The fruit is black, and the bunches compact, of the Norton type. Such a grape might be desirable to mix with Clinton or Norton.

The seedling secured from Mr. H. Beatty, Kelleys Island, Ohio, showed very high sugar for 1910, and low acid in both years. It has loose bunches, black fruit with white juice, is mild in flavor, and might be desirable for blending. Its parentage is unknown.

From Mr. J. Schonhardt, Venice, Ohio, two samples were obtained, numbered 8 and 9 in the table. No. 8 is like Concord in type of bunch and in color, but is very much higher in sugar content, with a moderately low acid. No. 9 is Catawba in type, with a remarkably high sugar and low acid. If these last have such growth characteristics as to render their culture practicable they can not fail to be of commercial value.

Sugar and acid content of seedlings or unknown varieties.

Seedling No.	Field No.	Grower and address.	Date of sampling.	Total sugar as invert.		Acid as tartaric.	
				Grams.	Per cent.	Grams.	Per cent.
1	195	N. Schneider, Vermilion, Ohio	Oct. 7, 1909	21.21	19.42	0.979	0.897
1	91	.....do	Sept. 24, 1910	20.00	18.40	1.144	1.053
1	204	.....do	Oct. 2, 1910	19.95	18.35	1.005	.925
2	122	E. L. Steuk, Venice, Ohio	Oct. 1, 1909	19.52	17.99	.521	.480
2	179	.....do	Oct. 6, 1909	17.41	16.21	.473	.440
2	241	.....do	Oct. 9, 1909	18.78	18.43	.450	.416
2	50	.....do	Sept. 16, 1910	19.96	17.42	.529	.418
2	131	.....do	Sept. 26, 1910	22.37	20.45	.439	.401
3	361	J. H. Bauman, Kelleys Island, Ohio	Oct. 20, 1909	20.43	18.76	.938	.861
4	491	L. C. Dodge, Middle Bass, Ohio	Oct. 27, 1909	13.44	12.64	1.301	1.223
4	53	.....do	Sept. 17, 1910	19.91	18.32	.743	.684
5	331	N. Wagner, Brownhelm, Ohio	Oct. 17, 1909	19.08	17.56	.754	.694
6	203	H. Kümmel, Middle Bass, Ohio	Oct. 7, 1909	18.37	16.90	.514	.473
6	69	.....do	Sept. 21, 1910	17.50	16.25	.664	.616
7	71	H. Beatty, Kelleys Island, Ohio	Sept. 24, 1909	18.41	17.05	.656	.607
7	348	.....do	Oct. 8, 1910	24.39	22.13	.626	.568
8	41	J. Schonhardt, Venice, Ohio	Sept. 16, 1910	22.43	20.45	.848	.763
9	229	.....do	Oct. 3, 1910	26.11	23.54	.683	.616

GRAPES GROWN IN VIRGINIA, 1908–1910.

Formerly Charlottesville, Va., was a grape-growing center of considerable importance, but the very destructive ravages of black rot from 1886 to 1890, before the present methods of controlling this disease were developed, among other factors resulted in greatly reducing the area of grapes in cultivation. There remains, however, a small but important industry which is confined chiefly to growing Norton grapes for wine in the Piedmont section of Virginia. This district is the natural home of the Norton, where, under careful cultivation, it grows to a perfection seldom reached elsewhere. With the exception of this variety, the grape-growing industry is of very little importance in the Virginia Piedmont. During the years 1909 and 1910, however, samples were obtained of all of the other varieties obtainable from the local wine cellars at Charlottesville.

The Norton is, when matured properly, such a valuable grape for red wine and for grape-juice manufacture that it is worthy of a thorough investigation. There is also every reason to believe that most of the commonly grown American grapes can be produced in the Virginia Piedmont of a quality quite equal to those of other sections, but the results show that at present this is not done. However, in the case of the Norton the high content of sugar and the low acid indicate a grape superior, in the main, to the samples of this variety from other districts.

The climate and soil conditions of the Virginia Piedmont are so different from those of the northern grape belt that it seems best to present the results for this district in a separate table. It is intended to extend the investigation of the composition of grapes grown in the Atlantic Coast States so as to cover the territory more thoroughly and to secure a larger number of samples. The data here presented are not sufficient to warrant conclusions, but they are interesting and show the possibility of producing a very high quality in at least some of the black grapes grown in this section.

The samples analyzed at the Stonehenge Laboratory were obtained through the courtesy of Mr. A. Russow, of the Monticello Wine Co., and were taken by him from the fruit brought to the wine cellar at vintage time. It was endeavored to make the sample represent the crop as delivered.

For 1908 only samples of Norton were obtained. These show a very high average sugar content and an average acid content of less than 1 per cent. This is certainly a high quality and warrants the statement that such a juice needs very little change in composition for wine making. The maximum sugar content found of 22.34 per cent, with 0.902 of total acid, is phenomenal for a strictly American grape. A comparison of the three years' results on Norton shows that the same vineyard has in each year yielded the maximum quality, the average for the three years being above 22 per cent of sugar and 8 per mille of acid (i. e., per thousand, or 0.8 per cent). In several instances the sampling of the Norton grapes covers a period of from four to seven days, yet there is no marked difference shown in composition. The question of the changes in percentage of acid and sugar in the grapes grown in this district needs a thorough investigation.

During 1909 and 1910 the sampling of several crops of Concord grapes also covered a period from four to seven days, and decided improvement was shown in some of these crops during this time, while in other cases it was slight. The sampling of several crops of Ives for like periods shows no decided results in favor of the later pickings, and in some cases a deterioration is indicated. These data are by no means to be accepted as proof that the crops actually deteriorated. The question needs a careful investigation of reserved vines, which can be studied for a much longer period, before it can be definitely answered. In the opinion of the writer none of the results for Concord and Ives show the quality which may be produced by thoroughly maturing these varieties. Table IV (p. 35) presents the average data obtained on these Virginia grapes.

TABULATION OF ANALYTICAL DATA ON SAMPLES COLLECTED AT SANDUSKY, OHIO.

DATA FOR 1908.

TABLE I.—*Average, maximum, and minimum data for 1908, arranged by varieties and localities. (Analyses by Hartmann and Eoff.)*

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
BACCHUS.															
Vermilion, Ohio	2	1	22.1	23.28	23.46	23.09	2.77	2.82	2.72	20.51	20.74	20.27	1.205	1.215	1.196
CATAWBA.															
Kelleys Island, Ohio	14	10	21.2	22.98	24.67	21.83	2.21	2.39	1.94	20.77	22.73	19.48	.825	1.013	.675
Middle Bass, Ohio	1	1	19.9	21.70	....	....	2.13	....	....	19.57	....	....	.743	....	....
North Bass, Ohio	1	1	19.3	20.89	....	....	2.09	....	....	18.80	....	....	.855	....	....
Port Clinton, Ohio	2	2	21.6	22.42	23.25	21.59	2.11	2.23	2.00	20.30	21.02	19.59	.769	.788	.750
Put in Bay, Ohio	6	6	21.1	22.74	24.57	21.59	2.23	2.70	1.87	20.51	22.70	19.57	.839	.971	.668
Vermilion, Ohio	1	1	19.1	20.41	....	....	2.00	....	....	18.41	....	....	.638	....	....
Averages, limits, and totals	25	21	20.9	22.64	24.67	21.59	2.19	2.70	1.87	20.45	22.73	19.48	.775	1.013	.668
CLINTON.															
Huron, Ohio	3	1	22.5	23.45	24.04	22.54	2.64	2.81	2.49	20.81	21.43	19.73	.975	1.125	.863
North Bass, Ohio	1	1	18.9	19.21	....	....	2.78	....	....	16.43	....	....	1.265	....	....
Vermilion, Ohio	1	1	19.7	20.07	....	....	2.56	....	....	17.51	....	....	1.215	....	....
Averages, limits, and totals	5	3	21.2	21.93	24.04	22.54	2.65	2.81	2.49	19.28	21.43	19.73	1.081	1.125	.865
CONCORD.															
Berlin Heights, Ohio	1	1	19.8	20.73	....	....	1.85	....	....	18.80	....	....	.473	....	....
Brownhelm, Ohio	1	1	18.2	18.74	....	....	1.68	....	....	17.06	....	....	.488	....	....
Kelleys Island, Ohio	2	2	19.5	20.57	20.83	20.31	1.90	2.29	1.51	18.67	19.32	18.02	.426	.473	.379
Marblehead, Ohio	1	1	17.2	18.16	....	....	1.21	....	....	16.95	....	....	.349	....	....
North Bass, Ohio	2	2	18.6	19.89	20.07	19.52	1.69	1.79	1.59	18.10	18.48	17.73	.488	.578	.398
Sandusky, Ohio	1	1	17.6	17.83	....	....	2.08	....	....	15.75	....	....	.454	....	....
Vermilion, Ohio	2	2	17.6	18.74	20.60	16.88	1.74	2.03	1.46	16.99	18.57	15.42	.495	.521	.469
Locality not given	1	1	16.7	16.70	....	....	1.40	....	....	15.30	....	....	.544	....	....
Averages, limits, and totals	11	11	18.3	19.12	20.83	16.88	1.72	2.29	1.46	17.40	19.32	15.42	.466	.578	.379
DELAWARE.															
North Bass, Ohio	2	1	24.8	27.27	28.46	26.09	2.03	2.28	1.78	25.24	26.18	24.31	.467	.495	.439
IONA.															
Vermilion, Ohio	1	1	21.6	23.30	....	....	2.21	....	....	21.09	....	....	.679	....	....
ISABELLA.															
Kelleys Island, Ohio	2	2	20.3	21.83	21.86	21.80	1.95	2.03	1.86	19.89	19.94	19.83	.540	.656	.424
IVES.															
Catawba Island, Ohio	1	1	16.6	17.64	....	....	1.80	....	....	15.84	....	....	.379	....	....
Ceylon, Ohio	4	3	17.0	18.37	18.66	16.13	1.87	1.98	1.75	15.49	16.91	14.28	.553	.608	.461
Danbury, Ohio	2	2	17.4	18.28	19.00	17.56	2.05	2.06	2.04	16.23	16.96	15.50	.506	.525	.488
Huron, Ohio	3	2	19.1	21.19	22.72	18.48	2.09	2.46	1.50	19.10	21.22	16.16	.490	.555	.375
Marblehead, Ohio	1	1	16.8	17.48	....	....	1.88	....	....	15.60	....	....	.514	....	....
Put in Bay, Ohio	2	1	17.2	18.03	18.14	17.93	2.09	2.40	1.78	15.94	16.36	15.53	.603	.776	.431
Vermilion, Ohio	6	6	16.7	17.16	19.68	15.71	1.96	2.11	1.79	15.19	17.74	13.62	.510	.551	.431
Averages, limits, and totals	19	16	17.9	18.09	22.72	15.71	1.97	2.46	1.50	16.12	21.22	13.62	.519	.776	.375

TABLE I.—*Average, maximum, and minimum data for 1908, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MONTEFIORE.															
Kelleys Island, Ohio	1	1	(1)	26.62	.....	.....	1.82	.....	.....	24.80	.....	.....	.401	.....	.....
NORTON.															
Huron, Ohio	1	1	25.4	27.17	.....	.....	3.05	.....	.....	24.12	.....	.....	1.013	.....	.....
Kelleys Island, Ohio	1	1	28.6	30.55	.....	.....	3.23	.....	.....	27.32	.....	.....	1.010	.....	.....
Venice, Ohio	1	1	25.4	27.38	.....	.....	3.00	.....	.....	24.38	.....	.....	.994	.....	.....
Averages, limits, and totals	3	3	26.5	28.37	.....	.....	3.09	.....	.....	25.27	.....	.....	1.006	.....	.....
RIESLING.															
Kelleys Island, Ohio	1	1	.....	20.65	.....	.....	2.08	.....	.....	18.57	.....	.....	.656	.....	.....
SCHRAIDT.															
North Bass, Ohio	1	1	.....	21.20	.....	.....	2.97	.....	.....	18.23	.....	.....	1.294	.....	.....
SEEDLING (SCHNEIDER).															
Vermilion, Ohio	1	1	25.9	28.17	.....	.....	2.33	.....	.....	25.84	.....	.....	.540	.....	.....

(1) Brix not read.

DATA FOR 1909.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities. (Analyses by Hartmann and Eoff.)*

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
AGAWAM.															
North East, Pa	1	1	16.7	17.69	.....	.....	2.15	.....	.....	15.54	.....	.....	1.043	.....	.....
Ripley, N. Y	1	1	(1)	22.07	.....	.....	1.81	.....	.....	22.26	.....	.....	.638	.....	.....
Averages, limits, and totals	2	2	[2] 16.7	19.88	.....	.....	1.98	.....	.....	17.90	.....	.....	.840	.....	.....
BACCHUS.															
Kelleys Island, Ohio	1	1	21.0	23.12	.....	.....	4.21	.....	.....	18.91	.....	.....	1.996	.....	.....
Middle Bass, Ohio	3	3	21.9	24.37	25.17	23.51	4.22	4.30	4.16	20.15	21.01	19.30	1.587	1.816	1.620
North Bass, Ohio	1	1	(1)	22.20	.....	.....	4.10	.....	.....	18.10	.....	.....	2.156	.....	.....
Sandusky, Ohio [3]	1	1	22.6	25.09	.....	.....	4.56	.....	.....	20.53	.....	.....	1.672	.....	.....
Brocton, N. Y	1	1	21.4	22.83	.....	.....	3.33	.....	.....	18.50	.....	.....	1.936	.....	.....
Averages, limits, and totals	7	7	[4] 21.8	23.76	25.17	23.51	4.12	4.30	4.16	19.64	21.01	19.30	1.852	1.816	1.620

(1) Brix not read.
[2] One sample.
[3] A. Schmidt, jr., & Bros.
[4] Only 6 samples.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
BRIGHTON.															
Put in Bay, Ohio	1	1	18.9	20.62	.....	.....	2.25	.....	.....	18.37	.....	.....	.653	.....	.....
Venice, Ohio	1	1	(1)	25.30	.....	.....	2.73	.....	.....	22.57	.....	.....	.611	.....	.....
Averages, limits, and totals	2	2	18.9	22.96	.....	.....	2.49	.....	.....	20.47	.....	.....	.632	.....	.....
CAMPBELL'S EARLY.															
Kelleys Island, Ohio	1	1	(1)	15.94	.....	.....	2.33	.....	.....	13.61	.....	.....	.784	.....	.....
CLINTON.															
Brownhelm, Ohio	2	1	[2] 22.1	21.95	24.59	19.31	4.23	4.26	4.21	17.72	20.38	15.05	1.935	2.130	1.740
Huron, Ohio	1	1	(1)	25.67	.....	.....	3.89	.....	.....	21.78	.....	.....	1.456	.....	.....
Middle Bass, Ohio	2	1	22.0	24.27	.....	.....	3.84	.....	.....	20.43	.....	.....	1.730	.....	.....
North Bass, Ohio	2	2	(1)	22.43	21.41	20.44	3.56	3.66	3.47	17.36	17.75	16.97	1.755	1.808	1.702
Sandusky, Ohio [3]	1	1	22.1	25.17	.....	.....	4.88	.....	.....	20.29	.....	.....	1.726	.....	.....
Sandusky, Ohio [4]	1	1	20.0	21.78	.....	.....	3.87	.....	.....	17.91	.....	.....	1.628	.....	.....
Venice, Ohio	4	2	[2] 18.7	18.92	19.97	17.83	4.05	4.26	3.82	14.87	15.76	14.01	1.568	1.932	1.309
North East, Pa	1	1	22.1	24.70	.....	.....	4.64	.....	.....	20.06	.....	.....	2.220	.....	.....
Bluff Point, N. Y	2	1	21.2	23.30	24.91	21.70	4.18	4.19	4.18	19.12	20.72	17.52	1.946	2.070	1.822
Brocton, N. Y	1	1	18.9	20.49	.....	.....	4.03	.....	.....	16.46	.....	.....	2.130	.....	.....
Averages, limits, and totals	17	12	20.9	22.07	24.91	17.83	4.08	4.26	3.47	17.80	20.72	14.01	1.777	1.808	1.309
CATAWBA.															
Brownhelm, Ohio	2	2	19.7	21.96	23.07	20.86	3.27	3.76	2.79	18.69	19.31	18.07	1.203	1.316	1.091
Danbury, Ohio	1	1	19.1	20.49	.....	.....	2.73	.....	.....	17.76	.....	.....	1.486	.....	.....
Euclid, Ohio	1	1	(1)	19.63	.....	.....	2.87	.....	.....	16.76	.....	.....	1.249	.....	.....
Kelleys Island, Ohio	7	7	19.3	20.99	22.25	19.34	2.90	3.66	2.41	18.09	19.31	16.93	1.278	1.384	1.163
Middle Bass, Ohio	9	5	[5] 19.7	21.13	23.20	19.60	2.49	2.82	2.32	18.63	20.88	17.15	1.033	1.174	.881
North Bass, Ohio	7	7	[6] 19.9	21.69	23.35	18.84	2.50	3.18	2.03	19.18	21.32	16.38	1.024	1.181	.844
Put in Bay, Ohio	17	13	[7] 19.0	20.83	22.80	18.48	2.70	3.32	2.11	18.14	19.68	15.97	1.096	1.335	.960
Sandusky, Ohio:															
American Wine Co	16	1	[8] 19.0	20.80	21.04	20.39	2.85	3.62	2.23	18.01	18.81	16.77	1.175	1.552	.945
Bass Island Vineyard Co	7	1	[9] 19.2	20.60	21.46	19.79	2.84	3.60	2.14	17.76	18.84	16.19	1.291	1.702	.866
Duroy & Haines	4	1	18.9	20.77	22.15	19.44	2.77	3.10	2.40	18.01	19.05	16.35	1.120	1.256	.968
Engels & Krudwig	1	1	20.3	22.16	.....	.....	3.58	.....	.....	18.57	.....	.....	1.226	.....	.....
A. Schmidt, jr., & Bros	3	1	17.2	18.27	19.00	17.09	2.15	2.17	2.13	16.12	16.86	14.92	1.113	1.181	1.058
Sweet Valley Wine Co.	3	1	18.0	19.18	21.15	18.01	2.33	2.47	2.16	16.86	18.68	15.85	1.190	1.238	1.163
Venice, Ohio	6	5	[10] 19.3	20.37	23.13	18.92	2.82	3.22	2.25	17.55	20.11	15.73	1.207	2.432	1.058
North East, Pa	3	3	17.4	19.01	19.60	18.40	3.01	3.21	2.89	16.00	16.66	15.51	1.527	1.642	1.410
Brocton, N. Y	2	2	16.8	17.86	19.26	16.47	3.07	3.55	2.59	14.79	16.67	12.92	1.792	1.972	1.612
Ripley, N. Y	1	1	19.2	21.07	.....	.....	2.29	.....	.....	18.78	.....	.....	1.144	.....	.....
Sheridan, N. Y	1	1	17.9	19.42	.....	.....	2.20	.....	.....	17.22	.....	.....	(11)	.....	.....
Averages, limits, and totals	91	54	19.0	22.80	23.35	16.47	2.72	3.76	2.03	17.91	20.11	12.92	1.167	2.432	.844
CONCORD.															
Brownhelm, Ohio	3	3	[2] 17.8	17.58	18.97	16.68	2.48	2.86	1.90	15.10	17.07	14.01	.841	.930	.698
Catawba Island, Ohio	1	1	16.9	17.75	.....	.....	2.03	.....	.....	15.72	.....	.....	.533	.....	.....
Danbury, Ohio	6	4	[2] 16.2	17.66	19.81	16.52	2.32	2.56	1.84	15.33	17.25	14.50	.672	.803	.555
Euclid, Ohio	1	1	(1)	19.79	.....	.....	2.73	.....	.....	17.42	.....	.....	.626	.....	.....
Gypsum, Ohio	1	1	17.9	18.19	.....	.....	2.30	.....	.....	15.89	.....	.....	.533	.....	.....
Kelleys Island, Ohio	4	2	[9] 18.8	20.07	21.81	19.08	2.16	2.85	1.81	17.91	19.99	16.49	.629	.776	.525
Middle Bass, Ohio	4	4	16.5	18.04	19.84	16.99	2.08	2.59	1.84	15.96	18.00	14.40	[12] .720	.....	.....
Noble, Ohio	1	1	(1)	18.08	.....	.....	2.66	.....	.....	15.42	.....	.....	.634	.....	.....
North Bass, Ohio	4	4	[2] 18.2	18.33	19.44	17.25	2.50	3.07	1.61	15.84	16.81	14.88	.752	1.050	.461

[1] Brix not read.
[2] One sample.
[3] Engels & Krudwig.
[4] A. Schmidt, jr., & Bros.
[5] Eight samples.
[6] Five samples.
[7] Sixteen samples.
[8] Twelve samples.
[9] Three samples.
[10] Four samples.
[11] Acid not made.
[12] One acid given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CONCORD—Continued.															
Port Clinton, Ohio	3	3	16.6	17.00	17.20	16.60	1.96	2.25	1.80	15.04	15.37	14.80	0.611	0.660	0.566
Put in Bay, Ohio	2	2	18.3	19.46	20.49	18.43	1.97	1.99	1.95	17.49	18.50	16.48	.645	.690	.600
Sandusky, Ohio:															
Bass Island Vineyard Co.	1	....	17.3	18.32	.....	.....	2.55	.....	.....	15.77	.....	.....	.739	.....	.....
Engels & Krudwig	1	....	17.0	18.32	.....	.....	2.66	.....	.....	15.66	.....	.....	.713	.....	.....
Sweet Valley Wine Co.	1	....	(1)	15.50	.....	.....	1.90	.....	.....	13.60	.....	.....	.930	.....	.....
Jno. Dorn	1	....	16.7	17.80	.....	.....	1.96	.....	.....	15.84	.....	.....	.724	.....	.....
Venice, Ohio	12	11	[2]17.8	18.74	21.23	16.65	2.39	2.79	1.74	16.35	18.44	14.22	[3].742	.968	.521
North East, Pa.	9	9	17.0	18.19	19.94	16.23	2.02	2.37	1.77	16.17	17.85	14.22	.874	1.076	.716
Bluff Point, N. Y	3	3	17.9	18.82	19.86	17.62	1.81	1.91	1.71	17.00	18.04	15.91	.729	.975	.488
Brocton, N. Y	2	2	17.3	18.39	18.56	18.22	2.06	2.29	1.84	16.32	16.72	15.93	.847	1.046	.649
Hammondsport, N. Y	2	2	16.7	17.77	17.93	17.62	1.79	1.80	1.78	15.98	16.13	15.84	.613	.645	.581
Keuka, N. Y	3	3	16.3	17.07	17.85	16.23	1.88	1.92	1.81	15.20	15.95	14.42	.753	.814	.705
Penn Yan, N. Y	1	1	16.7	17.64	.....	.....	2.15	.....	.....	15.49	.....	.....	.716	.....	.....
Pulteney, N. Y	1	1	15.5	15.84	.....	.....	1.80	.....	.....	14.04	.....	.....	.615	.....	.....
Ripley, N. Y	3	3	16.8	17.82	19.05	16.47	1.91	2.03	1.83	15.91	17.18	14.64	[4].827	.915	.739
Sheridan, N. Y	1	1	(1)	17.90	.....	.....	1.99	.....	.....	15.91	.....	.....	.829	.....	.....
Wayne, N. Y	2	2	16.9	17.80	17.80	17.80	1.70	1.79	1.61	16.10	16.19	16.01	.690	.735	.645
Westfield, N. Y	3	3	16.3	17.31	18.06	16.23	2.14	2.41	2.00	15.17	16.06	14.22	.893	.990	.799
Averages, limits, and totals	76	67	17.0	18.14	21.81	16.23	2.15	3.07	1.61	15.99	19.99	14.01	.748	1.076	.461
CYNTHIANA.															
Put in Bay, Ohio	1	1	(1)	24.14	.....	.....	5.04	.....	.....	19.10	.....	.....	1.598	.....	.....
DELAWARE.															
Brownhelm, Ohio	1	1	24.2	26.70	.....	.....	2.22	.....	.....	24.28	.....	.....	.705	.....	.....
Danbury, Ohio	1	1	(1)	19.44	.....	.....	2.70	.....	.....	16.65	.....	.....	.908	.....	.....
Euclid, Ohio	1	1	(1)	20.89	.....	.....	2.74	.....	.....	18.15	.....	.....	.803	.....	.....
Kelleys Island, Ohio	2	1	[5]22.1	24.25	24.30	24.20	2.21	2.45	1.97	22.04	22.23	21.85	.765	.878	.653
Middle Bass, Ohio	7	7	(1)	22.81	24.99	21.02	2.00	2.13	1.89	20.81	23.04	18.89	[4].773	.818	.728
Noble, Ohio	1	1	(1)	24.04	.....	.....	2.59	.....	.....	21.45	.....	.....	.791	.....	.....
North Bass, Ohio	5	5	[5]22.7	23.31	25.07	22.78	2.25	3.21	2.19	21.47	23.34	20.50	.823	1.031	.641
Sandusky, Ohio:															
Sweet Valley Wine Co.	2	1	21.3	23.09	25.43	20.45	2.32	2.46	2.19	20.76	23.24	18.29	.735	.739	.731
Venice, Ohio	2	2	(1)	25.21	27.12	23.20	2.86	2.89	2.84	22.34	24.28	20.41	.943	1.031	.855
North East, Pa.	2	2	21.8	24.07	25.49	22.65	2.55	2.67	2.43	21.54	22.82	20.22	1.022	1.061	.983
Bluff Point, N. Y	2	2	20.7	22.46	22.72	22.20	2.34	2.89	1.80	20.11	20.92	19.31	.758	.788	.728
Brocton, N. Y	1	1	20.2	22.17	.....	.....	2.94	.....	.....	19.23	.....	.....	1.016	.....	.....
Hammondsport, N. Y	3	3	20.5	21.99	22.86	21.04	1.96	2.10	1.85	20.03	20.92	19.19	.765	.803	.716
Penn Yan, N. Y	1	1	21.6	23.88	.....	.....	2.84	.....	.....	21.04	.....	.....	.934	.....	.....
Pulteney, N. Y	1	1	20.5	21.75	.....	.....	2.05	.....	.....	19.70	.....	.....	.881	.....	.....
Ripley, N. Y	1	1	18.4	19.05	.....	.....	1.95	.....	.....	17.10	.....	.....	.788	.....	.....
Sheridan, N. Y	1	1	21.0	22.93	.....	.....	2.20	.....	.....	20.73	.....	.....	.806	.....	.....
Wayne, N. Y	1	1	20.9	22.75	.....	.....	2.13	.....	.....	20.62	.....	.....	.821	.....	.....
Westfield, N. Y	1	1	17.3	18.56	.....	.....	2.36	.....	.....	16.20	.....	.....	.840	.....	.....
Averages, limits, and totals	36	34	20.9	22.97	25.49	20.45	2.36	3.21	1.80	20.61	24.28	19.19	.829	1.061	.641
DIANA.															
Kelleys Island, Ohio	1	1	20.1	22.07	.....	.....	2.17	.....	.....	19.90	.....	.....	.950	.....	.....
ELVIRA.															
Kelleys Island, Ohio	1	1	19.1	20.05	.....	.....	1.98	.....	.....	18.07	.....	.....	.765	.....	.....
Middle Bass, Ohio	4	4	(1)	16.08	17.85	14.64	2.25	2.45	1.98	13.82	15.87	12.40	.908	1.095	.656
North Bass, Ohio	2	2	(1)	16.41	18.32	14.51	2.51	3.25	2.78	13.40	15.07	11.13	1.277	1.354	1.200
Venice, Ohio	1	1	(1)	17.17	.....	.....	3.00	.....	.....	14.17	.....	.....	1.069	.....	.....
Averages, limits, and totals	8	8	19.1	16.66	18.32	14.51	2.50	3.25	1.98	14.29	15.87	11.13	1.002	1.354	.656

[1] Brix not read.
[2] Four samples.
[3] Ten acids given.
[4] Two acids given.
[5] On one sample.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ETTA.															
North Bass, Ohio	1	1	13.9	14.20	.....	.....	2.96	.....	.....	11.24	.....	.....	1.369	.....	.....
GREENS EARLY.															
Kelleys Island, Ohio	1	1	(1)	18.45	.....	.....	4.24	.....	.....	14.21	.....	.....	.758	.....	.....
GUT EDEL.															
Kelleys Island, Ohio	1	1	21.9	23.77	.....	.....	3.57	.....	.....	20.20	.....	.....	1.144	.....	.....
HAYS.															
Put in Bay, Ohio	1	1	(1)	20.94	.....	.....	2.79	.....	.....	18.45	.....	.....	.893	.....	.....
HARTFORD.															
Middle Bass, Ohio	1	1	19.1	19.60	.....	.....	2.04	.....	.....	17.56	.....	.....	(2)	.....	.....
IONA.															
Kelleys Island, Ohio	2	2	19.9	21.90	22.36	21.44	2.27	2.67	1.87	19.63	20.49	18.77	.806	.855	.758
Put in Bay, Ohio	1	1	19.3	21.83	.....	.....	3.02	.....	.....	18.81	.....	.....	.851	.....	.....
Brocton, N. Y	1	1	21.0	23.14	.....	.....	2.07	.....	.....	21.07	.....	.....	.881	.....	.....
Averages, limits, and totals	4	4	20.0	22.19	22.36	21.44	2.41	2.67	1.87	19.78	20.49	18.77	.836	.855	.758
ISABELLA.															
Euclid, Ohio	1	1	(1)	15.55	.....	.....	2.22	.....	.....	13.33	.....	.....	.821	.....	.....
Marblehead, Ohio	1	1	(1)	13.13	.....	.....	2.21	.....	.....	10.92	.....	.....	1.084	.....	.....
North East, Pa	1	1	14.9	15.94	.....	.....	2.44	.....	.....	13.50	.....	.....	1.140	.....	.....
Bluff Point, N. Y	2	2	16.6	17.60	17.67	17.54	2.09	2.16	2.02	15.51	15.52	15.51	.872	.874	.870
Brocton, N. Y	1	1	16.2	17.25	.....	.....	2.57	.....	.....	14.68	.....	.....	1.249	.....	.....
Averages, limits, and totals	6	6	16.1	16.18	17.67	17.54	2.27	2.16	2.02	13.91	15.52	15.51	1.008	.874	.870
IVES.															
Brownhelm, Ohio	3	2	[3] 17.5	18.73	19.44	17.64	3.05	3.45	2.53	15.68	15.99	15.11	.935	1.118	.720
Huron, Ohio	2	2	[3] 17.6	18.04	19.29	16.80	2.71	2.97	2.45	15.33	16.32	14.35	.637	.656	.619
Kelleys Island, Ohio	1	1	(1)	16.15	.....	.....	2.78	.....	.....	13.37	.....	.....	.896	.....	.....
Middle Bass, Ohio	2	2	16.7	17.78	19.00	16.57	2.53	2.79	2.28	15.25	16.21	14.29	.716	.784	.649
North Bass, Ohio	2	2	[3] 14.3	14.79	15.03	14.56	2.20	2.30	2.11	12.59	12.73	12.45	.742	.765	.720
Sandusky, Ohio:															
Engels & Krudwig	3	1	16.7	17.94	18.69	16.62	2.63	2.79	2.50	15.31	16.19	14.03	.651	.743	.525
Sweet Valley Wine Co.	2	1	17.0	18.66	18.71	18.61	3.29	3.62	2.96	15.37	15.65	15.09	.744	.780	.709
John Dorn	1	1	18.9	20.81	.....	.....	3.83	.....	.....	16.98	.....	.....	.788	.....	.....
A. Schmidt, jr., & Bros	1	1	18.8	20.28	.....	.....	3.11	.....	.....	17.17	.....	.....	.833	.....	.....
Put in Bay, Ohio	2	2	16.9	18.26	18.58	17.95	2.84	3.19	2.50	15.42	16.08	14.76	.697	.735	.559
Venice, Ohio	6	5	[4] 16.6	17.15	18.50	15.22	2.59	2.92	1.81	14.56	15.73	13.41	[5] .815	.904	.716
Vermilion, Ohio	3	2	[3] 17.4	17.30	17.75	16.47	2.30	2.73	2.04	15.00	15.71	13.74	.775	.844	.664
North East, Pa	1	1	12.6	13.39	.....	.....	2.22	.....	.....	11.17	.....	.....	.776	.....	.....
Hammondsport, N. Y	1	1	14.9	15.35	.....	.....	1.88	.....	.....	13.47	.....	.....	.683	.....	.....
Pulteney, N. Y	1	1	12.3	12.92	.....	.....	2.16	.....	.....	10.76	.....	.....	.904	.....	.....
Averages, limits, and totals	31	25	16.4	17.39	19.44	14.56	2.67	3.62	1.81	14.72	16.32	12.45	.768	1.118	.619
MARTHA.															
Brocton, N. Y	1	1	17.2	18.06	.....	.....	1.99	.....	.....	16.07	.....	.....	.840	.....	.....

[1] Brix not read.
[2] Acid not made.
[3] One sample.
[4] Two samples.
[5] Four acids given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MARION.															
North Bass, Ohio	1	1	23.1	25.17	.....	.....	4.40	.....	.....	20.77	.....	.....	1.718	.....	.....
MONTEFIORE.															
Kelleys Island, Ohio	2	1	[1] 18.0	21.21	23.35	19.08	2.68	3.22	2.16	18.52	20.13	16.92	.771	1.073	.469
Put in Bay, Ohio	1	1	(2)	24.54	.....	.....	3.27	.....	.....	21.27	.....	.....	.780	.....	.....
Venice, Ohio	1	1	(2)	24.54	.....	.....	3.04	.....	.....	21.51	.....	.....	.645	.....	.....
Averages, limits, and totals	4	3	18.0	22.88	23.35	19.08	2.92	3.22	2.16	19.96	20.13	16.92	.842	1.073	.469
NIAGARA.															
Brownhelm, Ohio	1	1	(2)	21.36	.....	.....	2.99	.....	.....	18.37	.....	.....	.713	.....	.....
Danbury, Ohio	1	1	(2)	15.63	.....	.....	2.66	.....	.....	12.97	.....	.....	.585	.....	.....
Euclid, Ohio	1	1	(2)	15.32	.....	.....	2.58	.....	.....	12.74	.....	.....	.551	.....	.....
Kelleys Island, Ohio	1	1	16.9	18.30	.....	.....	1.80	.....	.....	16.50	.....	.....	.585	.....	.....
Middle Bass, Ohio	1	1	(2)	18.74	.....	.....	3.17	.....	.....	15.57	.....	.....	.675	.....	.....
North Bass, Ohio	1	1	(2)	15.61	.....	.....	2.68	.....	.....	12.93	.....	.....	.581	.....	.....
North East, Pa	3	3	18.3	19.57	20.02	19.31	1.92	2.06	1.83	17.65	17.96	17.43	.781	.829	.746
Bluff Point, N. Y	2	1	16.1	16.74	17.93	15.55	1.48	1.80	1.16	15.76	16.13	14.39	.501	.664	.338
Brocton, N. Y	1	1	18.3	20.12	.....	.....	2.00	.....	.....	18.12	.....	.....	.690	.....	.....
Ripley, N. Y	3	3	16.6	17.72	18.16	17.33	1.84	1.93	1.80	15.88	16.23	15.53	[3] .665	.731	.599
Westfield, N. Y	1	1	15.7	16.44	.....	.....	3.30	.....	.....	13.14	.....	.....	.780	.....	.....
Averages, limits, and totals	16	15	17.1	17.93	20.02	15.55	2.22	2.06	1.16	15.72	17.96	14.39	.615	.829	.338
NORTON.															
Euclid, Ohio	1	1	(2)	25.85	.....	.....	3.34	.....	.....	22.51	.....	.....	1.456	.....	.....
Kelleys Island, Ohio	1	1	19.5	20.94	.....	.....	3.25	.....	.....	17.69	.....	.....	1.770	.....	.....
Middle Bass, Ohio	2	2	21.3	22.29	22.88	21.70	4.49	4.79	4.18	17.80	18.70	16.91	1.896	1.912	1.880
North Bass, Ohio	2	2	(2)	21.84	21.94	21.75	4.46	4.72	4.21	17.38	17.54	17.22	1.718	1.740	1.696
Put in Bay, Ohio	2	1	[1] 22.0	23.79	23.91	23.67	4.21	4.23	4.19	19.58	19.68	19.48	1.864	1.912	1.816
Sandusky, Ohio:															
Engels & Krudwig	1	1	25.4	27.96	.....	.....	6.13	.....	.....	21.83	.....	.....	1.996	.....	.....
Venice, Ohio	2	2	24.2	26.70	26.78	26.63	5.20	5.51	4.90	21.50	21.72	21.27	1.917	2.078	1.756
Averages, limits, and totals	11	10	22.5	24.00	26.78	21.70	4.49	5.51	4.18	19.50	21.72	16.91	1.812	2.078	1.696
POCKLINGTON.															
Put in Bay, Ohio	1	1	17.8	19.26	.....	.....	2.70	.....	.....	16.80	.....	.....	.926	.....	.....
Venice, Ohio	1	1	17.9	19.55	.....	.....	2.51	.....	.....	17.26	.....	.....	.656	.....	.....
Ripley, N. Y	1	1	(2)	18.19	.....	.....	1.72	.....	.....	16.15	.....	.....	.518	.....	.....
Averages, limits, and totals	3	3	17.8	19.00	.....	.....	2.31	.....	.....	16.85	.....	.....	.700	.....	.....
RIESLING.															
Brownhelm, Ohio	1	1	(2)	21.88	.....	.....	2.94	.....	.....	18.94	.....	.....	1.181	.....	.....
Kelleys Island, Ohio	2	2	19.8	21.61	22.65	20.57	2.42	2.47	2.38	19.18	20.18	18.19	.750	.825	.675
Averages, limits, and totals	3	3	19.8	21.70	22.65	20.57	2.59	2.47	2.38	19.13	20.18	18.19	.894	.825	.675
ROGERS.															
Brocton, N. Y	1	1	18.6	20.23	.....	.....	2.13	.....	.....	18.10	.....	.....	1.035	.....	.....
SALEM.															
North Bass, Ohio	1	1	(2)	19.21	.....	.....	1.60	.....	.....	17.61	.....	.....	.510	.....	.....

[1] One sample.

[2] Brix not read.

[3] Two acids given.

TABLE II.—*Average, maximum, and minimum data for 1909, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Average Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
SCHRAIDT.															
Middle Bass, Ohio	2	2	20.2	22.37	22.88	21.86	4.95	5.74	4.17	17.41	17.69	17.14	1.853	2.040	1.666
North Bass, Ohio	1	1	(1)	21.70	.....	.....	4.02	.....	.....	17.68	.....	.....	1.726	.....	.....
Averages, limits, and totals	3	3	20.2	22.15	22.88	21.86	4.64	5.74	4.17	17.50	17.69	17.14	1.826	2.040	1.666
UNKNOWN GRAPES.															
Brownhelm, Ohio	1	1	20.1	22.57	.....	.....	3.49	.....	.....	18.08	.....	.....	.754	.....	.....
Kelleys Island, Ohio	2	2	(1)	21.98	23.22	20.75	2.56	2.79	2.34	19.42	20.43	18.41	.797	.938	.656
Middle Bass, Ohio	2	2	17.0	18.42	20.36	16.49	2.52	3.05	1.99	15.91	18.37	13.44	.908	1.301	.514
North Bass, Ohio	1	1	20.3	21.10	.....	.....	3.71	.....	.....	17.39	.....	.....	1.365	.....	.....
Sandusky, Ohio:															
A. Schmidt, jr., & Bros.	1	....	19.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.866	.....	.....
Venice, Ohio	1	1	(1)	18.16	.....	.....	2.60	.....	.....	15.93	.....	.....	.679	.....	.....
Vermilion, Ohio	1	1	21.5	23.83	.....	.....	2.65	.....	.....	21.21	.....	.....	.979	.....	.....
Averages, limits, and totals	9	8	19.1	20.81	23.22	16.49	2.83	3.05	1.99	18.03	20.43	13.44	.898	1.310	.514
WILDER.															
Put in Bay, Ohio	1	1	16.1	16.96	.....	.....	2.52	.....	.....	14.44	.....	.....	1.020	.....	.....
EARLY WORDEN.															
Put in Bay, Ohio	1	1	(1)	19.68	.....	.....	2.90	.....	.....	17.02	.....	.....	.780	.....	.....
Venice, Ohio	1	1	17.8	19.31	.....	.....	2.04	.....	.....	17.47	.....	.....	.638	.....	.....
Averages, limits, and totals	2	2	17.8	19.49	.....	.....	2.47	.....	.....	17.24	.....	.....	.709	.....	.....
WOODRUFF RED.															
Middle Bass, Ohio	1	1	(1)	17.27	.....	.....	2.93	.....	.....	14.34	.....	.....	.409	.....	.....

[1] Brix not read.

DATA FOR 1910.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities.* (*Analyses by Eoff and Treuthardt.*)

[Results expressed in grams per 100 cc of expressed juice.]

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
AGAWAM.															
Fredonia, N. Y.	2	2	16.5	17.88	18.40	17.35	3.23	3.47	2.99	14.65	14.93	14.36	1.463	1.516	1.410
Geneva, N. Y.	1	1	17.9	19.16	.....	.....	2.62	.....	.....	16.54	.....	.....	1.106	.....	.....
Averages, limits, and totals	3	3	17.0	18.30	18.40	17.35	3.03	3.47	2.99	15.28	14.93	14.36	1.344	1.516	1.410
AMBER QUEEN.															
Geneva, N. Y.	1	1	17.6	18.63	.....	.....	2.61	.....	.....	16.02	.....	.....	1.436	.....	.....
AMERICA.															
Geneva, N. Y.	1	1	18.6	19.65	.....	.....	2.71	.....	.....	16.94	.....	.....	1.043	.....	.....
AMINIA.															
Geneva, N. Y.	1	1	17.5	18.50	.....	.....	1.82	.....	.....	16.68	.....	.....	.821	.....	.....
ADIRONDACK.															
Geneva, N. Y.	1	1	11.9	12.35	.....	.....	2.16	.....	.....	10.19	.....	.....	.911	.....	.....
BACCHUS.															
Kelleys Island, Ohio	1	1	23.1	25.38	.....	.....	3.27	.....	.....	22.11	.....	.....	1.714	.....	.....
North Bass, Ohio	1	1	24.5	16.07	.....	.....	3.34	.....	.....	12.73	.....	.....	1.826	.....	.....
Averages, limits, and totals	2	2	23.8	20.73	.....	.....	3.31	.....	.....	17.42	.....	.....	1.770	.....	.....
BARRY.															
Geneva, N. Y.	1	1	14.0	14.59	.....	.....	2.47	.....	.....	12.12	.....	.....	1.159	.....	.....
BELL.															
Geneva, N. Y.	1	1	20.1	21.78	.....	.....	3.00	.....	.....	18.78	.....	.....	1.114	.....	.....
BIG EXTRA 19.															
Geneva, N. Y.	1	1	16.5	17.01	.....	.....	3.10	.....	.....	13.91	.....	.....	1.466	.....	.....
BLACK EAGLE.															
Geneva, N. Y.	2	1	18.6	19.88	21.88	17.88	2.31	2.40	2.22	17.57	19.66	15.48	1.011	1.121	.900
BRIGHTON.															
Put in Bay, Ohio	1	1	17.8	18.90	.....	.....	2.50	.....	.....	16.40	.....	.....	.982	.....	.....
Venice, Ohio	3	1	[1]21.8	22.24	24.14	19.16	2.38	2.83	1.93	19.87	21.50	16.79	.713	.840	.630
Fredonia, N. Y.	1	1	17.1	18.40	.....	.....	2.19	.....	.....	16.21	.....	.....	.900	.....	.....
Geneva, N. Y.	1	1	18.7	19.92	.....	.....	2.19	.....	.....	17.73	.....	.....	.803	.....	.....
Pulteney, N. Y.	1	1	21.3	23.07	.....	.....	2.33	.....	.....	20.74	.....	.....	.934	.....	.....
Averages, limits, and totals	7	5	19.3	21.00	24.14	19.16	2.33	2.83	1.93	18.67	21.50	16.79	.822	.840	.630
BROWN.															
Geneva, N. Y.	1	1	15.8	16.36	.....	.....	2.72	.....	.....	13.64	.....	.....	1.106	.....	.....
BUTLER No. 1.															
Geneva, N. Y.	1	1	17.6	19.18	.....	.....	2.15	.....	.....	17.03	.....	.....	.833	.....	.....

[1] One sample.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CAMPBELL'S EARLY.															
Kelleys Island, Ohio	1	1	18.7	20.34	.....	.....	1.84	.....	.....	18.50	.....	.....	0.611	.....	.....
Middle Bass, Ohio	1	1	17.3	18.84	.....	.....	1.97	.....	.....	16.87	.....	.....	.641	.....	.....
Put in Bay, Ohio	1	1	15.2	15.74	.....	.....	2.28	.....	.....	13.46	.....	.....	1.042	.....	.....
Fredonia, N. Y	1	1	15.4	16.31	.....	.....	2.90	.....	.....	13.41	.....	.....	1.241	.....	.....
Averages, limits, and totals	4	4	16.7	17.81	.....	.....	2.25	.....	.....	15.56	.....	.....	.884	.....	.....
CARMAN.															
Geneva, N. Y	1	1	16.1	16.73	.....	.....	3.44	.....	.....	13.29	.....	.....	1.654	.....	.....
CATAWBA.															
Kelleys Island, Ohio	14	11	21.0	23.14	25.17	21.46	2.30	2.57	2.02	20.84	22.70	19.31	.999	1.170	0.840
Middle Bass, Ohio	28	22	21.1	23.39	24.99	21.78	2.46	3.02	1.86	20.93	22.55	19.25	1.063	1.305	.754
North Bass, Ohio	10	9	21.4	23.84	24.80	22.83	2.32	2.68	1.88	21.52	22.87	20.35	1.058	1.226	.795
Put in Bay, Ohio	10	9	20.7	22.82	24.57	20.23	2.52	2.73	2.27	20.30	22.01	17.96	1.192	1.335	1.095
Venice, Ohio	5	3	[1] 21.3	23.09	24.59	20.89	2.35	2.54	2.10	20.74	22.21	18.65	1.045	1.144	.938
Vermilion, Ohio	1	1	21.7	24.17	.....	.....	2.51	.....	.....	21.66	.....	.....	1.091	.....	.....
Sandusky, Ohio:															
Cook's Wine Cellar	16	1	20.7	22.85	23.41	21.99	2.62	3.56	2.18	20.23	20.88	18.43	1.091	1.219	.893
Bluff Point, N. Y	1	1	20.0	21.75	.....	.....	2.80	.....	.....	18.95	.....	.....	1.444	.....	.....
Jerusalem, N. Y	1	1	19.6	21.28	.....	.....	2.95	.....	.....	18.33	.....	.....	1.606	.....	.....
Pulteney, N. Y	1	1	21.3	23.38	.....	.....	2.60	.....	.....	20.78	.....	.....	1.230	.....	.....
Urbana, N. Y	4	4	20.3	22.31	23.38	20.81	2.71	2.92	2.30	19.60	20.65	17.89	1.286	1.530	.994
North East, Pa.:															
South Shore Wine Co.	2	1	18.0	19.25	19.26	19.23	3.15	3.49	2.81	16.20	16.42	15.77	1.729	1.996	1.462
Averages, limits, and totals	93	64	20.0	23.07	25.17	19.23	2.50	3.56	1.86	20.51	22.87	15.77	1.097	1.996	.754
CHAMPION.															
Geneva, N. Y	1	1	13.2	14.30	.....	.....	2.40	.....	.....	11.90	.....	.....	.915	.....	.....
CHAUTAUQUA.															
Geneva, N. Y	1	1	15.2	15.84	.....	.....	2.34	.....	.....	13.50	.....	.....	1.043	.....	.....
CLEVENER 7-12.															
Geneva, N. Y	1	1	17.8	18.71	.....	.....	3.75	.....	.....	14.96	.....	.....	1.196	.....	.....
CLINTON.															
North Bass, Ohio	1	1	23.5	26.01	.....	.....	3.68	.....	.....	22.33	.....	.....	1.520	.....	.....
Venice, Ohio	2	2	[2] 23.4	23.89	25.91	21.86	2.85	4.69	3.00	20.04	22.91	17.17	2.117	2.588	1.646
Geneva, N. Y	1	1	19.5	20.78	.....	.....	4.60	.....	.....	16.18	.....	.....	2.272	.....	.....
Pulteney, N. Y	1	1	24.1	26.46	.....	.....	3.26	.....	.....	23.20	.....	.....	1.726	.....	.....
North East, Pa.:															
South Shore Wine Co.	2	....	20.8	22.08	23.14	21.02	3.72	3.94	3.50	18.36	19.64	17.08	2.260	2.494	2.026
Averages, limits, and totals	7	5	22.3	23.60	.....	.....	3.81	.....	.....	19.79	.....	.....	2.039	.....	.....
CLOETA 19-2.															
Geneva, N. Y	1	1	([3])	18.92	.....	.....	2.90	.....	.....	16.02	.....	.....	1.088	.....	.....
COLERAIN.															
Geneva, N. Y	1	1	16.5	17.48	.....	.....	4.72	.....	.....	12.76	.....	.....	.870	.....	.....
CORBY.															
Geneva, N. Y	1	1	15.3	15.92	.....	.....	2.15	.....	.....	13.77	.....	.....	.938	.....	.....

[1] Four samples.

[2] One sample.

[3] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
CONCORD.															
Kelleys Island, Ohio	1	1	19.0	20.81	.....	.....	2.29	.....	.....	18.52	.....	.....	0.623	.....	.....
Middle Bass, Ohio	19	19	[1] 18.4	20.84	21.73	17.01	2.44	3.16	1.80	17.41	19.23	16.03	.801	1.069	0.611
North Bass, Ohio	3	1	19.6	21.90	22.30	21.20	2.27	2.59	2.03	19.63	20.17	19.01	.650	.671	.608
Venice, Ohio	6	5	[2] 20.5	22.43	23.43	21.59	2.65	3.50	1.96	19.76	20.37	18.88	.679	.836	.536
Vermilion, Ohio	1	1	17.3	18.19	.....	.....	2.52	.....	.....	15.67	.....	.....	.840	.....	.....
Brocton, N. Y	8	1	18.7	20.50	21.36	19.60	2.09	2.63	1.87	18.16	19.49	17.17	.786	.963	.604
Bluff Point, N. Y	[illegible]	1	16.5	17.77	.....	.....	2.59	.....	.....	15.18	.....	.....	.926	.....	.....
Fredonia, N. Y	[illegible]	1	16.5	17.69	19.37	15.16	2.41	2.65	2.03	15.10	16.72	12.67	.925	1.009	.795
Geneva, N. Y	1	1	14.7	15.42	.....	.....	2.46	.....	.....	12.96	.....	.....	1.050	.....	.....
Hammondsport, N. Y	2	2	16.5	17.71	18.66	16.75	2.53	2.56	2.49	15.18	16.10	14.26	.986	1.013	.960
Jerusalem, N. Y	1	1	18.6	20.15	.....	.....	2.25	.....	.....	17.90	.....	.....	.731	.....	.....
Pulteney, N. Y	1	1	18.7	19.60	.....	.....	2.41	.....	.....	17.19	.....	.....	.979	.....	.....
Silver Creek, N. Y	2	1	15.9	17.19	18.40	15.97	2.41	2.61	2.21	14.78	16.19	13.36	1.030	1.110	.949
Urbana, N. Y.:															
Urbana Wine Co	2	1	16.8	17.66	18.45	16.86	2.25	2.28	2.21	15.41	16.17	14.65	.974	.979	.968
North East, Pa	23	9	17.7	19.04	23.01	12.90	2.45	2.76	2.17	16.58	20.48	10.41	.905	1.136	.671
Lawton, Mich	6	2	18.9	20.37	20.99	19.13	2.40	2.66	2.21	17.97	18.75	16.81	.761	.945	.660
Averages, limits, and totals	88	48	18.0	19.42	23.43	12.90	2.40	3.50	1.80	17.05	20.48	10.41	.834	1.069	.536
CREVELING.															
Geneva, N. Y	1	1	15.4	16.18	.....	.....	2.25	.....	.....	13.93	.....	.....	1.009	.....	.....
CROTON 11-4.															
Geneva, N. Y	1	1	13.4	15.07	.....	.....	3.23	.....	.....	11.84	.....	.....	.788	.....	.....
DAISY 7-2.															
Geneva, N. Y	1	1	18.0	19.29	.....	.....	2.34	.....	.....	16.95	.....	.....	.653	.....	.....
DELAGO.															
Geneva, N. Y	1	1	18.6	19.86	.....	.....	2.61	.....	.....	17.25	.....	.....	1.009	.....	.....
DELAWARE.															
Kelleys Island, Ohio	1	1	24.7	27.51	.....	.....	2.52	.....	.....	24.99	.....	.....	.641	.....	.....
Middle Bass, Ohio	6	6	[3] 23.6	26.51	27.62	24.46	2.41	2.72	2.15	23.76	25.47	22.01	.841	.900	.765
North Bass, Ohio	1	1	23.5	26.25	.....	.....	1.68	.....	.....	24.57	.....	.....	.724	.....	.....
Venice, Ohio	3	3	[4] 24.3	26.77	27.54	25.30	2.48	2.92	2.21	24.29	25.24	23.09	.773	.881	.713
Bluff Point, N. Y	1	1	20.5	22.96	.....	.....	3.14	.....	.....	19.82	.....	.....	1.013	.....	.....
Fredonia, N. Y	3	1	18.8	20.42	21.99	19.63	2.95	3.21	2.75	17.47	19.24	16.42	1.229	1.312	1.076
Geneva, N. Y	1	1	18.8	20.15	.....	.....	2.41	.....	.....	17.74	.....	.....	1.166	.....	.....
Hammondsport, N. Y	3	3	21.0	22.87	23.12	22.49	2.46	2.62	2.37	20.41	20.60	20.12	.866	.945	.743
Pulteney, N. Y	3	2	20.4	21.97	23.96	19.86	2.38	2.52	2.26	19.59	21.70	17.50	.968	1.020	.919
Rheims, N. Y	1	1	20.1	21.52	.....	.....	2.41	.....	.....	19.11	.....	.....	1.020	.....	.....
Urbana, N. Y.:															
Urbana Wine Co	2	1	22.4	24.41	24.91	23.91	2.26	2.31	2.20	22.16	22.71	21.60	.857	.859	.855
New York State:															
Name lost	1	1	20.0	21.41	.....	.....	2.42	.....	.....	18.99	.....	.....	1.001	.....	.....
North East, Pa	2	1	20.8	22.38	22.72	22.04	2.61	2.71	2.51	19.77	20.01	19.53	.981	.994	.968
Lawton, Mich	1	1	22.6	25.20	.....	.....	2.54	.....	.....	22.66	.....	.....	.945	.....	.....
Averages, limits, and totals	29	24	21.8	23.92	27.62	19.63	2.49	3.21	2.15	21.36	25.47	16.42	.922	1.312	.713
DIAMOND.															
Geneva, N. Y	1	1	17.9	19.08	.....	.....	2.45	.....	.....	16.58	.....	.....	.998	.....	.....

[1] Eighteen samples.
[2] Four samples.
[3] Nine samples.
[4] Three samples.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
DIANA.															
North Bass, Ohio	1	1	21.5	23.70	.....	.....	2.35	.....	.....	21.35	.....	.....	0.728	.....	.....
Geneva, N. Y	1	1	19.6	21.23	.....	.....	2.66	.....	.....	18.67	.....	.....	1.200	.....	.....
Pulteney, N. Y	1	1	20.6	22.59	.....	.....	2.41	.....	.....	20.18	.....	.....	.923	.....	.....
Hammondsport, N. Y	1	1	21.3	23.57	.....	.....	2.68	.....	.....	20.89	.....	.....	1.009	.....	.....
Averages, limits, and totals	4	4	20.8	22.80	.....	.....	2.53	.....	.....	[illegible]	.....	.....	.965	.....	.....
DUCHESS.															
Geneva, N. Y	1	1	18.0	19.63	.....	.....	2.33	.....	.....	17.30	.....	.....	.750	.....	.....
Pulteney, N. Y	1	1	18.9	20.34	.....	.....	1.82	.....	.....	18.52	.....	.....	.638	.....	.....
Rheims, N. Y	1	1	17.8	18.87	.....	.....	2.24	.....	.....	16.63	.....	.....	.814	.....	.....
Averages, limits, and totals	3	3	18.3	19.61	.....	.....	2.13	.....	.....	17.48	.....	.....	.734	.....	.....
EARLY DAWN.															
Geneva, N. Y	1	1	17.6	18.56	.....	.....	2.34	.....	.....	16.22	.....	.....	1.065	.....	.....
EARLY GOLDEN 5-3.															
Geneva, N. Y	1	1	19.6	20.89	.....	.....	2.39	.....	.....	18.50	.....	.....	.848	.....	.....
EARLY VICTOR.															
Fredonia, N. Y	1	1	16.6	17.59	.....	.....	.....	.....	.....	8.07	.....	.....	.923	.....	.....
ELDORADO.															
Geneva, N. Y	1	1	20.3	22.02	.....	.....	2.39	.....	.....	19.63	.....	.....	.784	.....	.....
ELVIRA.															
Kelleys Island, Ohio	1	1	20.6	22.57	.....	.....	2.19	.....	.....	20.38	.....	.....	.754	.....	.....
Middle Bass, Ohio	1	1	16.5	17.14	.....	.....	2.72	.....	.....	14.42	.....	.....	1.050	.....	.....
North Bass, Ohio	1	1	17.4	18.92	.....	.....	1.46	.....	.....	17.46	.....	.....	.938	.....	.....
Venice, Ohio	1	1	17.8	19.05	.....	.....	2.68	.....	.....	16.37	.....	.....	.908	.....	.....
Hammondsport, N. Y	1	1	15.3	16.13	.....	.....	2.47	.....	.....	13.66	.....	.....	1.140	.....	.....
Pulteney, N. Y	1	1	16.1	16.70	.....	.....	2.50	.....	.....	14.20	.....	.....	1.264	.....	.....
Rheims, N. Y	1	1	15.5	15.79	.....	.....	2.78	.....	.....	13.01	.....	.....	1.462	.....	.....
Urbana, N. Y	1	1	17.4	18.74	.....	.....	2.69	.....	.....	16.05	.....	.....	1.144	.....	.....
Averages, limits, and totals	8	8	17.1	18.13	.....	.....	2.43	.....	.....	15.69	.....	.....	1.083	.....	.....
EMPIRE STATE.															
Put in Bay, Ohio	1	1	15.5	16.10	.....	.....	1.86	.....	.....	14.24	.....	.....	.848	.....	.....
Geneva, N. Y	1	1	18.7	20.18	.....	.....	2.23	.....	.....	17.95	.....	.....	.559	.....	.....
Averages, limits, and totals	2	2	17.1	18.14	.....	.....	2.05	.....	.....	16.10	.....	.....	.704	.....	.....
EUMELAN.															
North Bass, Ohio	1	1	14.9	15.58	.....	.....	2.04	.....	.....	13.54	.....	.....	.930	.....	.....
Geneva, N. Y	1	1	14.8	15.89	.....	.....	2.39	.....	.....	13.50	.....	.....	.964	.....	.....
Rheims, N. Y	1	1	19.8	21.20	.....	.....	2.15	.....	.....	18.87	.....	.....	.983	.....	.....
Urbana, N. Y.:															
Urbana Wine Co	2	1	20.2	21.58	21.75	21.41	2.17	2.20	2.13	19.42	19.62	19.21	.949	0.994	0.904
Averages, limits, and totals	5	4	18.0	19.13	21.75	21.41	2.18	2.20	2.13	16.95	19.62	19.21	.955	.994	.904
GOFF.															
Geneva, N. Y	2	1	16.8	18.07	21.67	14.46	2.43	2.51	2.35	15.64	19.32	11.95	.895	.964	.825

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
GOLD DUST.															
Geneva, N. Y.	1	1	17.1	18.24	.....	.....	2.53	.....	.....	15.71	.....	.....	0.694	.....	.....
GOLDEN.															
Geneva, N. Y.	1	1	17.4	18.69	.....	.....	1.65	.....	.....	17.04	.....	.....	.420	.....	.....
GLENFELD.															
Geneva, N. Y.	1	1	16.5	17.25	.....	.....	2.69	.....	.....	14.56	.....	.....	1.106	.....	.....
GUT EDEL.															
Kelleys Island, Ohio.	1	1	21.5	23.20	.....	.....	2.84	.....	.....	20.36	.....	.....	1.350	.....	.....
HAYES.															
Put in Bay, Ohio	1	1	16.8	17.72	.....	.....	2.56	.....	.....	15.16	.....	.....	1.118	.....	.....
HARTFORD.															
Geneva, N. Y.	1	1	19.3	20.83	.....	.....	2.67	.....	.....	18.16	.....	.....	.881	.....	.....
Fredonia, N. Y.	1	1	14.7	15.71	.....	.....	2.25	.....	.....	13.46	.....	.....	.904	.....	.....
Averages, limits, and totals	2	2	17.0	18.27	.....	.....	2.46	.....	.....	15.81	.....	.....	.893	.....	.....
HERBERT.															
Geneva, N. Y.	1	1	18.0	19.13	.....	.....	2.59	.....	.....	16.54	.....	.....	1.200	.....	.....
HEXAMER 24-20.															
Geneva, N. Y.	1	1	19.4	20.10	.....	.....	3.74	.....	.....	16.36	.....	.....	1.245	.....	.....
HICKS.															
Geneva, N. Y.	1	1	15.0	15.79	.....	.....	2.47	.....	.....	13.32	.....	.....	1.159	.....	.....
ILLINOIS CITY.															
Geneva, N. Y.	1	1	18.8	20.07	.....	.....	2.26	.....	.....	17.81	.....	.....	.998	.....	.....
IONA.															
Middle Bass, Ohio	1	1	(1)	23.17	.....	.....	2.39	.....	.....	20.78	.....	.....	.664	.....	.....
North Bass, Ohio	2	2	21.5	24.03	25.07	22.99	2.47	2.61	2.32	21.57	22.46	20.67	.904	1.155	0.653
Vermilion, Ohio	2	2	21.5	23.67	23.88	23.46	2.36	2.38	2.34	21.31	21.50	21.12	.928	.956	.900
Geneva, N. Y.	1	1	19.6	21.25	.....	.....	2.83	.....	.....	18.42	.....	.....	1.426	.....	.....
Portland, N. Y.	1	1	21.5	23.83	.....	.....	2.46	.....	.....	21.37	.....	.....	1.046	.....	.....
Pulteney, N. Y.	1	1	21.4	23.41	.....	.....	2.51	.....	.....	20.90	.....	.....	1.234	.....	.....
Averages, limits, and totals	8	8	21.2	23.38	25.07	22.99	2.48	2.38	2.32	20.90	22.46	20.67	1.004	1.155	.653
ISABELLA.															
Kelleys Island, Ohio	1	1	19.1	21.04	.....	.....	2.26	.....	.....	18.78	.....	.....	.716	.....	.....
North Bass, Ohio	1	1	20.0	21.83	.....	.....	2.12	.....	.....	19.71	.....	.....	.836	.....	.....
Hammondsport, N. Y.	1	1	20.0	21.88	.....	.....	2.11	.....	.....	19.77	.....	.....	.773	.....	.....
Pulteney, N. Y.	1	1	16.7	17.88	.....	.....	2.44	.....	.....	15.44	.....	.....	1.016	.....	.....
Urbana, N. Y.	4	4	16.6	17.79	19.58	16.31	2.50	2.74	2.31	15.29	16.97	13.57	1.028	1.076	.956
Wayne, N. Y.	1	1	17.1	18.56	.....	.....	2.67	.....	.....	15.89	.....	.....	1.163	.....	.....
Averages, limits, and totals	9	9	17.6	19.15	19.58	16.31	2.40	2.74	2.31	16.75	16.97	18.57	.967	1.076	.956

[1] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ISABELLA SEEDLING.															
Geneva, N. Y.	1	1	15.6	16.47	.....	.....	2.84	.....	.....	13.63	.....	.....	1.174	.....	.....
IVES.															
Ceylon, Ohio	3	3	18.4	19.34	20.55	18.45	2.43	2.52	2.32	16.92	18.23	16.03	0.678	0.844	0.559
Danbury, Ohio	1	1	15.4	16.62	.....	.....	2.71	.....	.....	13.91	.....	.....	.608	.....	.....
Kelleys Island, Ohio	1	1	17.5	18.63	.....	.....	2.09	.....	.....	16.54	.....	.....	.555	.....	.....
Middle Bass, Ohio	9	8	16.4	17.41	19.31	14.93	2.47	2.73	2.23	16.92	16.68	12.37	.823	.960	.713
Venice, Ohio	2	2	[1] 18.3	18.66	19.81	17.51	2.77	2.95	2.59	15.89	16.86	14.92	.733	.746	.720
Vermilion, Ohio	2	2	20.1	21.36	21.44	21.28	2.45	2.55	2.35	18.91	18.93	18.89	.655	.683	.626
Bluff Point, N. Y.	1	1	19.6	21.33	.....	.....	2.48	.....	.....	18.85	.....	.....	.641	.....	.....
Hammondsport, N. Y.	1	1	17.9	19.42	.....	.....	2.52	.....	.....	16.90	.....	.....	.836	.....	.....
North East, Pa.: South Shore Wine Co.	2	1	16.3	17.20	18.22	16.18	3.02	3.08	2.96	14.18	15.14	13.22	1.063	1.151	.975
Averages, limits, and totals	22	20	17.3	18.42	21.44	14.93	2.54	3.08	2.23	15.88	18.23	12.37	.772	1.151	.559
JANESVILLE.															
Geneva, N. Y.	1	1	16.6	17.48	.....	.....	3.53	.....	.....	13.95	.....	.....	1.462	.....	.....
LINDLEY.															
Fredonia, N. Y.	1	1	15.5	16.13	.....	.....	2.69	.....	.....	13.44	.....	.....	1.155	.....	.....
Geneva, N. Y.	1	1	18.8	20.26	.....	.....	2.49	.....	.....	17.77	.....	.....	.953	.....	.....
Averages, limits, and totals	2	2	17.2	18.20	.....	.....	2.59	.....	.....	15.61	.....	.....	1.054	.....	.....
LUCILE.															
Fredonia, N. Y.	1	1	15.6	16.15	.....	.....	2.70	.....	.....	13.45	.....	.....	.915	.....	.....
Geneva, N. Y.	1	1	(2)	17.59	.....	.....	2.12	.....	.....	15.47	.....	.....	.814	.....	.....
Averages, limits, and totals	2	2	[1] 15.6	16.87	.....	.....	2.41	.....	.....	14.46	.....	.....	.865	.....	.....
MARION.															
Geneva, N. Y.	2	1	19.0	21.07	21.15	20.99	3.77	3.83	3.70	17.31	17.32	17.29	1.566	1.610	1.522
MASSASOIT.															
Geneva, N. Y.	1	1	16.3	17.09	.....	.....	1.88	.....	.....	15.21	.....	.....	.754	.....	.....
MILLS.															
Portland, N. Y.	2	1	20.0	21.99	22.07	21.91	2.14	2.15	2.13	19.85	19.94	19.76	.741	.754	.728
MISSOURI RIESLING.															
Kelleys Island, Ohio	1	1	19.4	20.78	.....	.....	2.42	.....	.....	18.36	.....	.....	.941	.....	.....
MONTEFIORE.															
Kelleys Island, Ohio	1	1	(2)	23.65	.....	.....	2.29	.....	.....	21.36	.....	.....	.784	.....	.....
MOORE DIAMOND.															
Fredonia, N. Y.	1	1	18.0	19.52	.....	.....	3.06	.....	.....	16.46	.....	.....	1.121	.....	.....
MOORE EARLY.															
Fredonia, N. Y.	1	1	15.1	15.87	.....	.....	.....	.....	.....	9.04	.....	.....	.825	.....	.....

[1] One sample.

[2] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
MOYER.															
Fredonia, N. Y	1	1	18.5	20.00	.....	.....	2.50	.....	.....	17.50	.....	.....	0.780	.....	.....
Geneva, N. Y	1	1	19.1	20.12	.....	.....	2.40	.....	.....	17.72	.....	.....	.641	.....	.....
Averages, limits, and totals	2	2	18.8	20.06	.....	.....	2.45	.....	.....	17.61	.....	.....	.711	.....	.....
R. W. MUNSON.															
Geneva, N. Y	1	1	14.7	15.48	.....	.....	2.91	.....	.....	12.57	.....	.....	1.490	.....	.....
NECTAR.															
Geneva, N. Y	1	1	18.3	19.79	.....	.....	2.53	.....	.....	17.26	.....	.....	1.256	.....	.....
NIAGARA.															
North Bass, Ohio	2	1	19.2	21.08	21.54	20.62	2.17	2.30	2.03	18.92	19.24	18.59	.448	0.450	0.446
Benton, N. Y	1	1	15.6	16.91	.....	.....	2.21	.....	.....	14.70	.....	.....	.754	.....	.....
Fredonia, N. Y	1	1	15.3	16.02	.....	.....	3.13	.....	.....	12.89	.....	.....	.968	.....	.....
Geneva, N. Y	1	1	12.9	13.42	.....	.....	2.06	.....	.....	11.36	.....	.....	.773	.....	.....
Penn Yan, N. Y	1	1	12.6	14.38	.....	.....	2.71	.....	.....	11.67	.....	.....	.840	.....	.....
North East, Pa.: South Shore Wine Co	1	1	17.8	19.08	.....	.....	2.37	.....	.....	16.71	.....	.....	.806	.....	.....
Lawton, Mich	1	1	17.0	18.03	.....	.....	2.13	.....	.....	15.90	.....	.....	.791	.....	.....
Averages, limits, and totals	8	7	16.2	17.50	21.54	20.62	2.37	2.30	2.03	15.13	19.24	18.59	.728	.....	.....
NOAH.															
Geneva, N. Y	1	1	16.5	17.33	.....	.....	3.08	.....	.....	14.25	.....	.....	1.632	.....	.....
NORFOLK.															
Geneva, N. Y	1	1	17.4	18.58	.....	.....	2.48	.....	.....	16.10	.....	.....	1.144	.....	.....
NORTON.															
Kelleys Island, Ohio	1	1	22.8	24.27	.....	.....	3.53	.....	.....	20.74	.....	.....	1.856	.....	.....
North Bass, Ohio	1	1	23.9	26.54	.....	.....	3.65	.....	.....	22.89	.....	.....	1.522	.....	.....
Venice, Ohio	2	2	(1)	24.16	24.20	24.12	3.54	3.59	3.49	20.62	20.63	20.61	1.787	1.942	1.632
Vermilion, Ohio	1	1	23.5	26.01	.....	.....	3.85	.....	.....	22.16	.....	.....	1.462	.....	.....
Averages, limits, and totals	5	5	23.4	25.43	24.20	24.12	3.62	3.59	3.49	21.41	20.63	20.61	1.683	1.942	1.632
OLITA.															
Geneva, N. Y	1	1	17.0	18.16	.....	.....	2.19	.....	.....	15.97	.....	.....	.908	.....	.....
PIERCE.															
Geneva, N. Y	1	1	14.0	14.87	.....	.....	2.45	.....	.....	12.42	.....	.....	1.253	.....	.....
POCKLINGTON.															
North Bass, Ohio	1	1	17.8	19.16	.....	.....	1.87	.....	.....	17.29	.....	.....	.596	.....	.....
Venice, Ohio	1	1	18.0	19.31	.....	.....	2.53	.....	.....	16.78	.....	.....	1.095	.....	.....
Averages, limits, and totals	2	2	17.9	19.24	.....	.....	2.20	.....	.....	17.04	.....	.....	.846	.....	.....
REGAL.															
Portland, N. Y	1	1	16.8	18.14	.....	.....	2.03	.....	.....	16.11	.....	.....	.836	.....	.....
REQUA.															
Geneva, N. Y	2	1	17.9	19.23	20.70	17.75	2.27	2.36	2.18	16.75	18.52	14.97	.844	.960	.728
ROGERS NO. 24.															
Geneva, N. Y	1	1	15.0	15.66	.....	.....	2.47	.....	.....	13.19	.....	.....	1.238	.....	.....

[1] Brix not read.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
ROGERS NO. 32.															
Geneva, N. Y	1	1	16.9	17.85	.....	.....	2.42	.....	.....	15.43	.....	.....	1.181	.....	.....
RUPERT.															
Geneva, N. Y	1	1	16.1	16.83	.....	.....	3.55	.....	.....	13.28	.....	.....	1.756	.....	.....
SALEM.															
North Bass, Ohio	1	1	20.5	22.49	.....	.....	1.58	.....	.....	20.91	.....	.....	.521	.....	.....
Bluff Point, N. Y	1	1	18.9	20.60	.....	.....	2.78	.....	.....	17.82	.....	.....	.938	.....	.....
Pulteney, N. Y	1	1	22.2	24.41	.....	.....	2.63	.....	.....	21.78	.....	.....	1.024	.....	.....
Averages, limits, and totals	3	3	20.5	22.50	.....	.....	2.33	.....	.....	20.17	.....	.....	.828	.....	.....
ST. LOUIS.															
Geneva, N. Y	1	1	15.2	15.76	.....	.....	2.45	.....	.....	13.31	.....	.....	1.140	.....	.....
SCHRAIDTES.															
Middle Bass, Ohio	1	1	19.7	21.15	.....	.....	3.86	.....	.....	17.29	.....	.....	1.632	.....	.....
North Bass, Ohio	1	1	20.9	23.20	.....	.....	3.93	.....	.....	19.27	.....	.....	1.556	.....	.....
Averages, limits, and totals	2	2	20.3	22.18	.....	.....	3.90	.....	.....	18.28	.....	.....	1.594	.....	.....
TELEGRAPH.															
Fredonia, N. Y	1	1	16.3	17.46	.....	.....	3.56	.....	.....	13.90	.....	.....	1.256	.....	.....
TRIUMPH.															
Geneva, N. Y	1	1	(1)	16.88	.....	.....	2.79	.....	.....	14.09	.....	.....	1.354	.....	.....
UNKNOWN.															
Kelleys Island, Ohio	1	1	23.8	26.43	.....	.....	2.04	.....	.....	24.39	.....	.....	.626	.....	.....
Middle Bass, Ohio	3	2	[3] 18.9	21.23	22.54	20.05	2.49	2.63	2.30	18.74	19.91	17.50	.677	0.743	0.623
Venice, Ohio	3	2	23.6	26.02	28.49	24.38	2.39	2.77	2.01	23.84	26.11	22.37	.657	.848	.439
Vermilion, Ohio	3	1	20.3	22.66	22.72	22.59	2.68	2.72	2.64	18.23	20.00	14.75	1.075	1.144	1.005
Fredonia, N. Y	1	1	16.1	17.62	.....	.....	3.65	.....	.....	13.97	.....	.....	1.354	.....	.....
Averages, limits, and totals	11	7	21.0	23.11	28.49	20.05	2.58	2.77	2.01	20.55	26.11	14.75	.813	1.144	.439
TRAGEDY.															
Geneva, N. Y	1	1	(1)	18.82	.....	.....	2.17	.....	.....	16.65	.....	.....	.776	.....	.....
VERGENNES.															
Geneva, N. Y	1	1	18.1	19.50	.....	.....	2.72	.....	.....	16.78	.....	.....	1.163	.....	.....
Portland, N. Y	2	1	18.4	19.92	20.26	19.58	2.31	2.50	2.11	17.62	18.15	17.08	1.041	1.129	.953
Averages, limits, and totals	3	2	18.3	19.78	.....	.....	2.44	.....	.....	17.34	.....	.....	1.082	.....	.....
WAPENUKA.															
Geneva, N. Y	1	1	12.9	13.47	.....	.....	4.05	.....	.....	9.42	.....	.....	1.084	.....	.....
WILDER.															
Fredonia, N. Y	1	1	15.6	16.49	.....	.....	2.38	.....	.....	14.11	.....	.....	1.189	.....	.....
WORDEN.															
Venice, Ohio	10	2	[2] 18.3	19.33	22.51	15.08	2.34	2.95	2.00	16.99	20.28	12.13	.876	1.283	.634
Fredonia, N. Y	1	1	12.8	13.49	.....	.....	2.54	.....	.....	10.95	.....	.....	1.372	.....	.....
Geneva, N. Y	3	1	13.8	14.68	14.80	14.56	2.45	2.53	2.36	12.56	13.21	12.03	1.271	1.312	1.211
Penn Yan, N. Y	1	1	13.1	14.20	.....	.....	2.27	.....	.....	11.93	.....	.....	1.020	.....	.....
Averages, limits, and totals	15	5	14.3	17.88	.....	.....	2.36	.....	.....	16.46	.....	.....	.998	.....	.....

[1] Brix not read. [2] Two samples. [3] One sample.

TABLE III.—*Average, maximum, and minimum data for 1910, arranged by varieties and localities*—Continued.

Variety and locality.	Number of samples.	Number of growers.	Brix.	Total solids.			Sugar-free solids.			Sugar as invert.			Acid as tartaric.		
				Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
WYOMING RED.															
Fredonia, N. Y.	1	1	15.9	16.78	.....	.....	2.95	.....	.....	13.83	.....	.....	0.855	.....	.....
Geneva, N. Y.	1	1	14.1	14.85	.....	.....	2.09	.....	.....	12.76	.....	.....	.754	.....	.....
Averages, limits, and totals	2	2	15.0	15.82	.....	.....	2.52	.....	.....	13.29	.....	.....	.805	.....	.....
YONKEN HONEY DEW.															
Geneva, N. Y.	1	1	17.9	19.10	.....	.....	2.76	.....	.....	16.34	.....	.....	.956	.....	.....
No. 3197	1	1	19.6	22.36	.....	.....	2.26	.....	.....	20.10	.....	.....	.758	.....	.....
No. 4069	1	1	15.7	16.68	.....	.....	1.72	.....	.....	14.96	.....	.....	.604	.....	.....
No. 2698	1	1	15.2	15.87	.....	.....	1.56	.....	.....	14.31	.....	.....	.510	.....	.....
No. 98	1	1	19.3	20.83	.....	.....	1.79	.....	.....	19.04	.....	.....	.544	.....	.....
No. 116	1	1	15.8	16.68	.....	.....	1.83	.....	.....	14.85	.....	.....	.529	.....	.....
No. 3345	1	1	13.6	14.09	.....	.....	1.87	.....	.....	12.22	.....	.....	.731	.....	.....
No. 2717	1	1	17.0	18.08	.....	.....	1.89	.....	.....	16.19	.....	.....	.645	.....	.....
No. 2652	1	1	19.9	21.31	.....	.....	2.06	.....	.....	19.25	.....	.....	.645	.....	.....

TABULATION OF ANALYTICAL DATA ON SAMPLES GROWN AND ANALYZED AT CHARLOTTESVILLE, VA.

TABLE IV.—*Average, maximum, and minimum data, 1908–1910, arranged by varieties. (Analyses by Hartmann and Eoff.)*

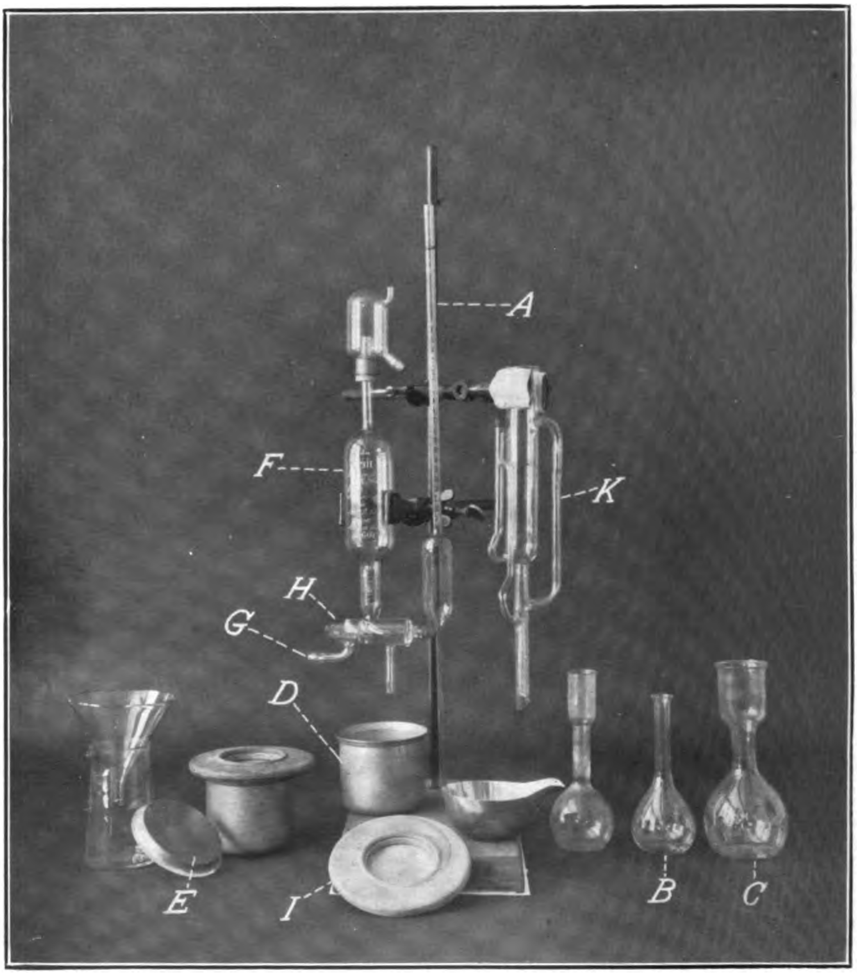
[Results expressed in grams per 100 cc of juice.]

Year.	Variety.	Number of samples.	Number of growers.	Specific gravity.	Total solids.			Sugar-free solids.			Total sugar as invert.			Acid as tartaric.		
					Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Maximum.	Minimum.
1908	Norton	9	7	1.1009	26.26	27.88	24.46	3.64	4.50	2.97	22.63	24.73	19.99	1.088	1.290	0.975
1909	Concord	14	5	1.0731	18.94	20.49	16.99	2.99	3.54	2.59	16.01	17.50	14.08	.744	.840	.645
1909	Cynthiana	1	1	1.0918	23.91	.....	.....	4.16	.....	.....	19.75	.....	.....	.986	.....	.....
1909	Delaware [1]	2	....	1.0969	25.30	26.43	24.17	2.86	3.08	2.63	22.45	23.80	21.09	.702	.784	.619
1909	Elvira	1	1	1.0698	18.11	.....	.....	2.76	.....	.....	15.35	.....	.....	.735	.....	.....
1909	Ives	12	7	1.0702	18.26	22.09	15.92	3.15	3.54	2.79	15.11	18.60	13.00	.753	.866	.653
1909	Noah	2	1	1.0876	22.80	22.88	22.72	2.75	2.75	2.74	20.06	20.13	19.98	.957	.990	.923
1909	Norton	11	9	1.0957	24.93	25.88	23.49	3.56	3.98	2.92	21.35	22.45	20.00	.925	1.001	.776
1909	Riesling	1	1	1.0647	16.99	.....	.....	2.69	.....	.....	14.30	.....	.....	.851	.....	.....
1910	Concord [2]	12	5	1.0747	19.44	21.65	17.04	3.11	3.61	2.60	16.33	18.15	14.07	.823	.904	.724
1910	Cynthiana	1	1	1.0962	25.04	.....	.....	3.92	.....	.....	21.10	.....	.....	1.080	.....	.....
1910	Delaware [1]	1	....	1.0888	23.12	.....	.....	2.12	.....	.....	21.00	.....	.....	.551	.....	.....
1910	Ives	15	9	1.0714	18.60	20.23	15.00	3.09	4.74	2.31	15.38	16.85	12.69	.714	.908	.611
1910	Norton	23	10	1.1003	26.23	28.54	24.17	3.88	4.63	3.37	22.44	25.04	20.10	.957	1.328	.750

[1] Mixed crops.

[2] Three samples from Crozet, Va.

O



APPARATUS FOR BEET ANALYSIS.

A, Sucrose pipette. *B*, Sugar flask. *C*, Flask for cold or hot water digestion. *D*, *E*, *I*, Metal cups and covers for the Sachs Le Docte method. *F*, Automatic pipette for the Sachs Le Docte method. *K*, Modified Soxhlet extractor for alcohol extraction method.

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ANALYSES OF SUGAR BEETS, 1905 TO 1910,

TOGETHER WITH

METHODS OF SUGAR DETERMINATION.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., June 14, 1911.

Sir: I have the honor to submit for your approval a report prepared in the sugar laboratory of this bureau on the analyses of sugar beets made during the years 1905 to 1910, inclusive, together with methods of determining the percentage of sugar in the beet. This compilation, representing, as it does, beets from many different localities, will be useful in answering the many inquiries received as to the quality of beets grown in the various sections of the country and the best methods for determining their sugar content. I recommend, therefore, that the manuscript be published as Bulletin No. 146 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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ANALYSES OF SUGAR BEETS, 1905 TO 1910,

TOGETHER WITH

METHODS OF SUGAR DETERMINATION.

INTRODUCTION.

Many requests are received at this department for reports on analyses of beets grown in different localities and also for information as to the fitness of certain sections for growing beets. Many inquiries are also received as to the best methods for the analysis of beets. This report, including a résumé of the analyses made of beets grown in various sections of this country throughout a period of six years, and also a statement of the methods used for beet analysis, with a discussion of their comparative accuracy, has been compiled to meet this demand.

As early as 1862 [1] the Department of Agriculture became interested in sugar-beet work, and for the past 30 years this bureau has been engaged in the analysis of sugar beets and in making investigations for the improvement of this industry. The following bulletins have been published giving the results of this work: No. 3*, The Northern Sugar Industry, 1883; No. 5*, The Sugar Industry of the United States, 1885; No. 27*, The Sugar-Beet Industry, 1890; Nos. 30*, 33*, 36*, 39*, and 52*, devoted to experiments with sugar beets in the years 1890, 1891, 1892, 1893, and 1897; Nos. 64, 74, 78, 95, and 96, devoted to a five years' study of the influence of environment upon the composition of the sugar beet, undertaken in 1900 to 1904, inclusive.

The bulletins marked with an asterisk (*) are out of print and are not available for distribution; the others may be had on application. Also, as a part of Progress of the Beet Sugar Industry of the United States, published yearly as a special report of the Secretary of Agriculture, there has been issued in the years 1897, 1898, 1899, and 1900 a report covering the analytical figures obtained in the analyses of samples of beets for these years. Since 1900 there has been no publication of results of analyses of beets made in the Bureau of Chemistry other than those published in the study of the influence of environment on the composition of the beet. Many samples have been analyzed since that time.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 52, p. 12.

During the years 1901–1904 many samples of beets were analyzed for local sugar projects, but their results are not included. The results from 1905 on to 1910 have been tabulated by States and counties. The location of the county in the State is noted by the usual sign, namely: □ center of State; □- east of center of State; ⟋□ southwest of center of State, etc. The figures given are for the average weight of beets (expressed in ounces), per cent of sugar in juice, and purity of the juice (the per cent of sugar in the solids of the juice). The condition of the sample as received is also stated. In this table no averages for States or counties are given, as so many factors enter into the results that conclusions based on such data might be misinterpreted. In fact, one can only form a comparative opinion as to the fitness of a certain region for beet growing from these analyses. The reasons for this may be briefly stated and serve also to illustrate the need for very careful work before passing final judgment on the possibilities of any section for beet culture.

FACTORS TO BE CONSIDERED IN INTERPRETING ANALYTICAL RESULTS.

A sugar beet is a plant that is greatly influenced by environment, cultivation, etc. Beets from the same seed may be grown in the same soil and under the same climatic conditions and the sugar contents at maturity be very different, owing to different methods of cultivation. A farmer who has grown beets a number of years in succession may raise a better crop than one in the same locality who has had no experience. Many of the samples herein reported came from farmers who had never raised beets before; some of the crops were no doubt raised on land entirely unsuitable for beet culture, and the results from the latter experiments would be of value as a test only if the soil of the plat were representative of this particular section. The large number of persons applying for beet seeds during a year made it impossible to keep a detailed record of the varying cultural and climatic conditions, although instructions were sent in all cases regarding the selection of the plat for the work and cultivation methods.

Correct sampling methods are also important to insure comparable results. Many people believe that the more prolific the growth of a beet the higher will be the percentage of sugar, and so select large beets from a patch for testing. This is not true, however, except in rare cases. Again, the sugar content varies with the degree of ripeness. As a beet matures the percentage of sugar increases; so selection with regard to this point is important. Two persons going through a field for sampling may draw samples that will vary as much as 3 or 4 per cent in sugar content. But if one is familiar with the work, the results from 20 or even fewer beets may fairly represent the field at that particular stage of growth. As no attention was paid to the method of sampling in over 90 per cent of the cases here reported, the results, as a basis for judging of the suitability for a given area for beet cultivation, are apt to be misleading.

Another factor which may affect the amount of sugar found is the condition of the beets when received for analysis. The healthy beet when taken from the ground is crisp, but on exposure to air and heat it soon loses moisture and becomes wilted; the next step is rotting. In losing water, the percentage of sugar present will, of course, be increased, but this increase is seldom in the same ratio as the loss in weight. Experiments conducted in 1891[1] at the Schuyler station in Nebraska show what may happen under these conditions. Beets were dug on October 3, carefully cleaned, and the leafy tops removed. They were then placed in the sun and reweighed at the end of each 24 hours. The daily temperature was 68° F. and the mean maximum 90° F. A heavy wind was blowing most of the time. Beginning with 152 pounds of beets, the loss in weight after one day was 13.2 per cent, after two days 23.8 per cent, after three days 32.4 per cent, and after four days 37.5 per cent. A sample drawn from the fresh beets showed 15.1 per cent of sugar, while at the end of the fourth day a sample drawn showed 17.1 per cent, an increase of only 2 per cent of sugar. Calculating the original sugar content of 15.1 per cent for the loss in weight, the beets should contain at the end of the four days 24.2 per cent, showing a loss of 7.1 per cent in sugar in the beet. This is a remarkable loss, but the experiment was carried on under severe weather conditions, great heat and wind.

A second trial was made in which clean beets were divided into three portions of 25 pounds each. One portion was left in the field, another was kept in the air but under a shed where the direct rays of the sun did not come in contact with the beets, while the third portion was analyzed. At the end of three days there was a 20 per cent loss in weight for the shed beets and a 22 per cent decrease for the field beets. The sugar content of the fresh beets was 16.2 per cent, of the shed beets 19.6 per cent, and of the field beets 18.3 per cent. Figuring the loss in weight as moisture, the sugar percentage of the shed beets should be 20.2 per cent at the end of the three days and of the field beets 20.7 per cent. The sugar losses here noted are not so large as in the previous experiment, being only 0.6 per cent when beets were kept away from the direct rays of the sun, but 2.4 per cent when they were placed in the sun.

Other experiments that have been tried indicate that after harvesting sun and heat work great detriment to the sugar content. An interesting experiment, showing that under certain conditions the sugar content does increase in proportion to the loss in weight, was tried at the same station. Twenty beets of about the same size and of the same degree of ripeness were selected. Ten were analyzed immediately and the other 10 were wrapped in oiled paper and sent to Washington for examination. The results are given in the following table:

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[1] U. S. Dept. Agr., Bureau of Chemistry, Bul. 36, p. 62.

Comparative analyses before and after shipment.

Time of analysis.	Loss in weight.	Per cent of sugar in beets.	Grams of sugar in beets.
	Per cent.		
Fresh	....	14.7	47.9
After shipment to Washington	12	16.6	47.9

There was a loss in weight during shipping of 12 per cent, but an increase of 1.9 per cent in the beet is noted, and the actual amount of sugar in the beet remains unchanged.

In the case of the samples herein reported the directions for shipping beets to Washington for analysis were to wrap each beet separately in oil paper, this paper being sent the grower, together with the shipping tag. In many cases the beets were fresh when received, but in a large percentage of instances they had evidently dried out before wrapping them for sending. It is important in using the analytical data contained in the tables to remember that these factors may have influenced the results.

For commercial purposes a beet of over 12 per cent sugar content, 12.5 to 13.5 per cent of sugar in the juice, and at least 80 per cent purity, and which weighs over 1 pound but under 4 or 5 pounds, is considered the most desirable. The contracts of most sugar companies with the farmers are based on these figures, but at times deviations from them are accepted. With these facts in mind one may form an opinion of the manufacturing value of the beets reported in the tabulated data (see p. 23).

METHODS OF SAMPLING.

The accuracy of the determination of sugar in the beet depends largely upon mechanical processes and accurate results are difficult to obtain unless a stated procedure is strictly followed, thus eliminating many chances of error. As with many other procedures, the statement of methods for the analysis of sugar beets is not sufficiently detailed and its limitations are not pointed out, so that an inexperienced worker may obtain accurate results. For example, all methods for beet analysis presuppose a finely divided pulp. With some methods the pulp must be much finer than with others. This is especially true of "instantaneous" methods or those obtaining the sugar by digestion in the cold. The sampling and subsampling of the beets are important details, for unless the sample is representative the results obtained are of little value.

SAMPLING FROM THE FIELD.

The beet chemist is often called upon to take a sample from the field. This sample should accurately represent the whole crop to be of any value as an index of the condition of the field. There are many methods of accomplishing this, some of a mathematical nature. The method to be employed depends somewhat upon the size of the field and the purpose for which the results are to be used. If the plat is small and it is the purpose to sample often, then a smaller sample may be used, but when the fields are extensive a larger sample should be drawn.

As an example of a mathematical method of sampling, the following is given: Look over the plat, noting the stand, and remove all beets that are not up to the average condition of the field. Go through again and dig every fourth to tenth beet (according to size of plat) for the analytical sample; every twentieth beet should be taken if the plat is very large. From 20 to 100 beets should constitute a sample. Another method is to remove all the beets in one row or a measured portion of a row and use these as the analytical sample. Care must be exercised that the row selected is representative.

To ascertain for factory purposes whether or not the crop is ripe and ready for harvest, the general practice is to walk across the field from one corner to the opposite one, noting the stand and the general condition of the beets as indicated by their leaves, general appearance, etc.; then on returning to dig from 5 to 20 beets at random, selecting those in average condition. Here the judgment of the sampler is wholly relied upon, but with experience and a knowledge of the variety under cultivation, a representative sample should be obtained.

Whatever method is used, it is essential in digging and cleaning the beets not to injure the skin and not to break the roots too short. The topping should consist only in removing the leaves, the crown being left in place for the chemist to remove; this prevents drying of the sample, etc.

SAMPLING FROM PILE, WAGON, OR CAR.

In sampling from a pile instead of from a field the judgment of the sampler again must be relied upon. A good procedure is to examine the pile of beets, noting the general shape and average size, and then to select from 10 to 50 beets for analysis, depending on the size of the pile. A proportionate number of large, small, and irregular-shaped beets should be used as they occur in the pile.

In drawing samples from a wagonload or carload of beets, it is customary to adopt one of the following procedures: When a dumping wagon is used, a bushel basket is placed on the bin in the path of the falling beets and withdrawn after the load is dumped. The beets in the basket constitute the analytical sample. If an ordinary wagon is used a bushel basket full of the beets is forked out during the unloading. In case the sample is taken directly from the cars, three or more samples should be selected from different portions of the car as it is being unloaded in the same manner as from the ordinary wagon.

LABORATORY SAMPLING AND PULPING.

The sampling of beets in the laboratory is a very important matter, as for the final analysis only a very small portion is used. The samples offered for analysis are composed of from 10 to 100 beets, and in an ordinary laboratory it is practically impossible to pulp all of the larger sample; therefore, one must resort to removing a section from each beet and pulping this. The removal of the right section is a difficult matter, since the sugar is not evenly distributed throughout the beet. Many persons have studied the localization of the sugar content of the beet. Prominent among them are Violette, Wiesner, De Vries, and Marek.[1] The latter went into the subject quite thoroughly in 1882. Proskowetz a number of years later worked out the localization of the sugar content in the beet as follows: By dividing a beet into four or five equal horizontal sections, as is shown in figure 1, he obtained the following percentages of sugar in the various portions:

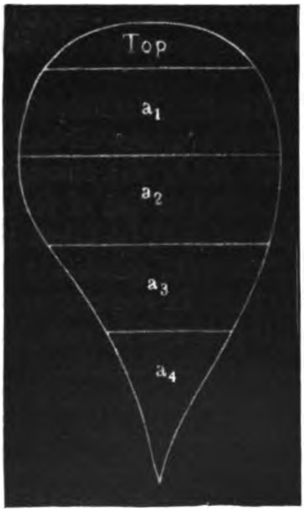

FIG. 1.—Horizontal sectioning of beets.

Percentages of sugar in horizontal sections of the beet.

Part of beet.	Sample 1.	Sample 2.	Sample 3.
In top....	12.30	14.08	17.52
A_1........	12.62	14.36	17.82
A_2........	12.57	14.42	17.68
A_3........	11.95	14.50	17.26
A_4........	(1)	14.20	(1)

1 Only four sections made.

From this it is shown that the bulged section (A_1) has the highest percentage of sugar. Other observers by dividing the beet into more and smaller sections obtained the same result, namely, that the highest sugar content is located in the region of the point of gravity of the beet mass and diminishes on both sides toward the top and the tip. Dividing the beet as is shown in figure 2—that is, cutting it into circular portions from the center to the skin—the following results were obtained:

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[1] Within the last few months Floderer and Herke have reviewed the previous work on this subject and have also added a large amount of careful experimental work. They come to practically the same conclusions as are here given. Osterr.-Ung. Zts. Zuckerind. Land., 1911, 40: 385.

Percentage of sugar in different vertical sections of the beet.

Section of beet.	Per cent sugar.
B_1...........	13.99
B_2...........	14.12
B_3...........	14.13
B_4...........	12.98

From these data it is seen that the zone next to the outside of the beet contains the highest sugar per cent, the next inside zone being nearly as high, while the outside and the central zones are lower. If the beet were cut into more zones, it would be found that the highest sugar occurred in a zone about three-fourths of the way from center to the outside.

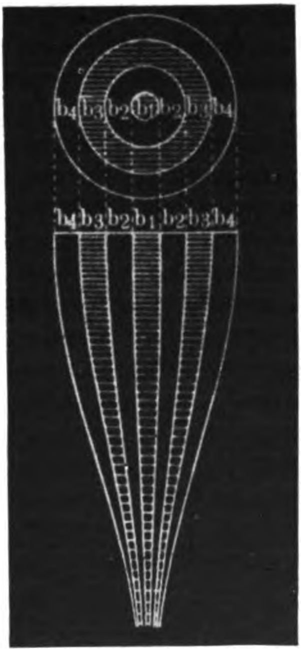

FIG. 2.—Vertical sectioning of beets.

Plant physiology shows that some cells contain much more sugar than others. If the beet is perfect in form a section taken throughout its complete length and passing to the center of the beet would be representative. If the beet is not of even form an error is apt to creep in, as it has been found that the per cent of sugar is higher in the portion of the beet that is depressed than in the well-expanded portions. If the weights of the sections removed in sampling could be so regulated that they bore an exact ratio to the whole weight of the beet, and this were constant for all, then these sections would accurately represent the large sample. Unfortunately this condition can only be approached, as it is possible to cut a beet in half or even in quarters, but beyond that it is difficult to subdivide by hand. The hand method is the one usually employed, however, in sampling beets, although machines in the form of cone-shaped rasps, which remove a section from the beets, are manufactured. The rasp is circular in form, thicker at the center and tapering to the edge. By running a beet over this fast-revolving rasp, the section is removed in the form of a fine pulp, and may be caught in a basket or box placed below it. When using from 20 to 50 beets the sample of ground pulp is quite large, and for accurate work it must be rapidly mixed and quartered, drawing one or more samples for analysis. In mixing it is necessary to guard against evaporation and also squeezing of the pulp; evaporation causes, of course, a higher sugar content than is naturally present, while squeezing may result in either a higher or lower sugar content, depending on the sample drawn. With quick mixing, however, little loss occurs.

In case the section has been removed by hand, it must be reduced to a pulp. There are many machines made for this purpose; in general, any meat chopper which yields a finely divided product may be used, but some forms are preferable to others. An evenly cut or shredded pulp should be obtained by grinding with little or no pressing. Machines that operate by feeding the beets by means of a screw to the cutting knife are very liable to express some of the juice, while if the screw is fitted with knives which do the cutting as well as the feeding the pressure on the pulp is lessened. Other machines have a revolving bowl with rotatory knives constantly turning, the two being driven by the same power. This yields a fine pulp with little pressing, but is hardly capable of receiving halves or even quarters of beets to be sliced. An ordinary horse-radish grater serves admirably for reducing sections of beets to a fine pulp. This grater has a rotating drum provided with nails extending from its surface about a quarter of an inch. An adjustable chute extends to the drum, so that the beets can be fed to it by hand. The nails may be replaced by embedding hacksaw blades in the drum and allowing the coarse teeth to stand out above the surface. In using such a grater care must be exercised not to feed too fast and to have the carrier for the beets just touch the points of the drum. In this way the beet is cut squarely off and no portion goes through uncut. It is highly important to feed beets into this machine in such a way that the grater comes in contact with the skin of the beet first, otherwise it is difficult to shred the skin, which is generally very tough. When one sample is finished all of the beet particles must be carefully brushed off of the drum and machine to avoid mixing the samples. This, however, is true with any machine. After shredding, the sample may be thoroughly mixed by hand.

SAMPLING AND TESTING SEED BEETS.

Beets that are to be used for seed production are tested for sugar content, and it is essential to obtain a sample from them without injuring their productiveness. This is usually accomplished by boring a hole through the beet in some such way as is shown in figure 3, and collecting the borings for analysis. It is highly important to control the direction of the passage of this rasp through the beet. As has been shown, the sugar is not equally distributed throughout the beet. It behooves one, therefore, to cut through as many zones as possible and the same cut should be made in all cases if the results are to be comparable. Much work has been done to determine the point of sampling and it has been shown that the rasp should enter near the edge of the leaf scar and take a direction at an angle of 45° to the main axis through the beet. This can be accomplished by having the beet placed on a moving board tilted at the proper angle and pressed against the rasp, or the rasp may be pressed against the beet. Rasps are manufactured which remove this core whole, or collect the cuttings in the shaft of the drill (fig. 4). The sample obtained this way seldom amounts to 26 grams and it is difficult to press this quantity and obtain sufficient juice to determine the dry substance and also the sugar. The per cent of sugar is determined direct by one of the instantaneous methods or by the hot-water digestion method.[1] Ten grams of the beet pulp may be digested, made up to 38.7 cc; and polarized in a 200 mm tube; the reading will be the per cent of sugar. Or 6.48 grams of the cuttings may be digested, made up to 50 cc, and a reading made in a 400 mm tube. Again, the beet cuttings in the rasp can be placed in a tared dish and the whole weighed. The pulp is then washed into a 100 cc flask if the sample amounts to about 13 grams, or into a 200 cc flask if it is nearly 26 grams, and a water digestion is made in the usual way. The per cent of sugar obtained, multiplied by the relation of the weight of sample used to 26 grams gives the percentage of sugar present. A correction should be made for the space occupied by the marc, 26 grams of pulp occupying 0.6 cc.

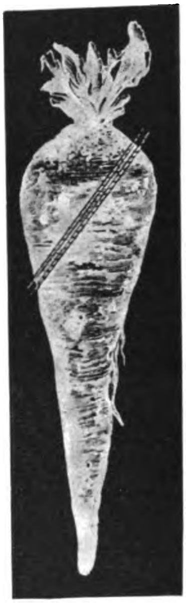

FIG. 3.—Correct position of boring rasp in beet.

The basic lead acetate solution is practically the only one used for clarifying beet solutions for polarization. The clarifying power of this solution depends somewhat upon its basicity. Browne[2] has shown that as the basicity increases the polarization of a raw cane sugar or cane sirup increases. As large an increase will hardly take place in the polarization of a beet, but it shows that care must be taken in the preparation of the basic lead acetate solution, "Three well-defined subacetates have been prepared by the digestion

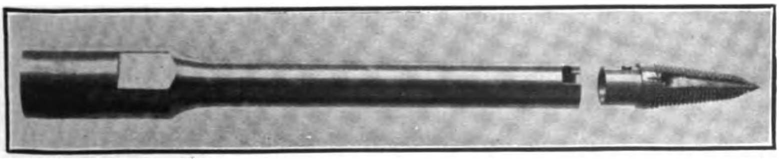

FIG. 4.—Boring rasp.

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[1] See pages 18 and 19.

[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 223.

of litharge with normal lead acetate. These are 3PbAc2PbO the one usually prescribed for clarification, PbAcPbO the monobasic, and PbAc2PbO the diabasic."[1] Variations in time of digestion, in quantities of litharge used, and in temperature of digestion will result in solutions containing mixtures of all of these.

To prepare this reagent boil 3 parts (by weight) of neutral lead acetate and 1 part of yellow litharge with 10 parts of water until the reaction is completed or the material is practically all dissolved. This generally takes not over half an hour. Cool and dilute the solution with water to a specific gravity of 1.25 or 53.7 Brix. The solution is filtered or allowed to stand until clear. The bottle should be kept tightly corked, as the composition of the solution changes. It may also be prepared by dissolving dry lead subacetate (containing 72.81 per cent of lead) in water until the specific gravity of the solution is 1.25 or 53.7 Brix.

METHODS FOR DETERMINING SUGAR.

Methods for the determination of sugar in the beet may be divided into two general classes, namely, direct and indirect. The former may be subdivided again according to the solvent used and the temperature of extraction. There is only one indirect method and this will be considered first.

INDIRECT METHOD.

The indirect method depends on pressing the juice from the beets, determining the sugar in the juice, and then by a factor calculating the per cent of sugar in the beet. In practice this is accomplished by inclosing the beet cuttings in a jute or cotton cloth, placing the whole in a press, and catching the juice in a vessel large enough to hold it all. The juice is thoroughly mixed, poured into a cylinder, and allowed to stand until the air bubbles have collected at the surface; generally from 20 to 30 minutes is necessary. This foam is brushed aside when the Brix hydrometer is placed in the liquid to obtain a reading on the content of solids. The hydrometer is allowed to come to rest before a reading is made, and should float free of the sides during the reading. After observing the reading and noting the temperature of the solution, the normal or double normal amount of the juice is either drawn up in a sucrose pipette (Spencer's method, *A*, frontispiece) or is weighed. This portion is run into a 100 cc flask with water, and a solution of basic lead acetate added in a sufficient quantity to produce a complete precipitation, but an excess is to be avoided. The flask shown at *B*, frontispiece, is an excellent shape for sugar analysis, because the slanting sides prevent air bubbles from remaining on its surface. The flask is then filled to the mark with water, shaken, filtered, and polarized. The percentage of sugar in the juice thus obtained is multiplied by a factor to obtain the amount of sugar in the beet. This factor corrects for the marc or solid portion of the beet. It is not, however, accurate to determine the marc by the usual method, namely, by washing away the soluble portion of the beet, drying, and weighing, calculating the per cent of solids, subtracting this from 100, and using the figure found for the juice factor. With this procedure the results will be too high for the reason that the marc contains water other than that holding the sugar and no correction is made for it. The marc of beets varies from 4 to 5.5 per cent, with an average of about 4.7 per cent. The juice factor calculated from this figure would be about 95.3, but when the colloidal water or water of marc is considered the figure is from 88 to 95, depending on the condition of the beets and the pressure used. A factor as low as 82 may be obtained if the beets are badly wilted from long standing in a dry climate, such as Colorado. This factor may be determined for a given locality by selecting beets of average condition and finding the per cent of sugar in the juice by the indirect method just given; then on a small portion of the same sample of beets determining the per cent of sugar in the beet by one of the direct methods, and dividing the per cent of sugar in the beet by the per cent of sugar in the juice. Such a factor is applicable to beets of like condition.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 122, p. 223.

The indirect method is used somewhat extensively, for by its use in addition to the percentage of sugar the purity of the juice (the per cent of sugar in the solids of the juice), which is considered by some to be an important factor in beet analysis, is obtained. This method is advantageous in that large quantities of pulp, representing many beets, can be used for pressing; therefore it is very easy to obtain an average sample when the original quantity is large. The main objection to it is that it does not give the sugar in the beet direct, the use of a factor being necessary. This average factor varies somewhat in different localities, ranging from 91.5 to 93, and its use works to the advantage of one sample and to the disadvantage of another. If not much depends on the accuracy of the results, this method can be used, but it can hardly be recommended when exactness is desired.

ERRORS OF THE METHOD.

Errors in the actual determination of the percentage of sugar creep in from the following sources, but with beets of 15 or even 20 per cent sugar content these errors may be within the error of reading or the limits of the sensibility of the instrument:

(1) Use of the wrong normal weight. Most polariscopes that have been purchased during the last five or six years are standardized for

26 grams of sugar, dissolved and made up to 100 true cubic centimeters with water at 20° C., and the polarization made in a 200 mm tube at the same temperature. The old normal weight of 26.048 grams of sugar was for 100 Mohr cubic centimeters, and probably the polarization was to be made at 17.5° C.

(2) Use of the wrong flask, namely, Mohr cubic centimeter flasks in place of true cubic centimeter flasks or vice versa.

(3) Use of old polariscopes whose accuracy has not been checked. A polariscope scale can be checked against pure sugar, but ordinarily it is much better to check against a standardized quartz plate.

(4) Use of sucrose pipette in place of a normal weight. This pipette can be easily standardized and the error due to this source checked, but in general it is better to obtain the normal or double normal amount of juice by weighing.

An error that may amount to several per cent can be introduced by using the same jute cloth for pressing beets when the per cents of sugar in the two samples vary greatly. A large error may also enter from dilution by not removing all of the water used in washing out the press after each sample. Errors in reading the Brix hydrometer are sometimes due to air in the juice and also to the adherence of the hydrometer to the sides of the cylinder. If these chances of error are guarded against, the reading of the per cent of sugar in the juice should be accurate. The following table[1] shows the possible error from the use of a wrong factor:

Variations in results obtained by using different factors.

Sugar in juice.	Sugar in beet calculated by factor—					
	90	91	92	93	94	95
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
12	10.8	10.92	11.04	11.16	11.28	11.40
13	11.7	11.83	11.96	12.09	12.22	12.35
14	12.6	12.74	12.88	13.02	13.16	13.30
15	13.5	13.65	13.80	13.95	14.10	14.25
16	14.4	14.56	14.72	14.88	15.04	15.20
17	15.3	15.47	15.64	15.81	15.98	16.15
18	16.2	16.38	16.56	16.74	16.92	17.10
19	17.1	17.29	17.48	17.67	17.86	18.05
20	18.0	18.20	18.40	18.60	18.80	19.00
21	18.9	19.11	19.32	19.53	19.74	19.95

Should the juice polarize 20° and a factor of 90 be used when 95 is the correct one, the resulting figure for the sugar in the beet is just 1 per cent too low. If the results by this method are used as a basis for the price paid for beets, the grower would lose the price of an extra per cent of sugar per ton, which varies from 20 to 25, or 33 cents.

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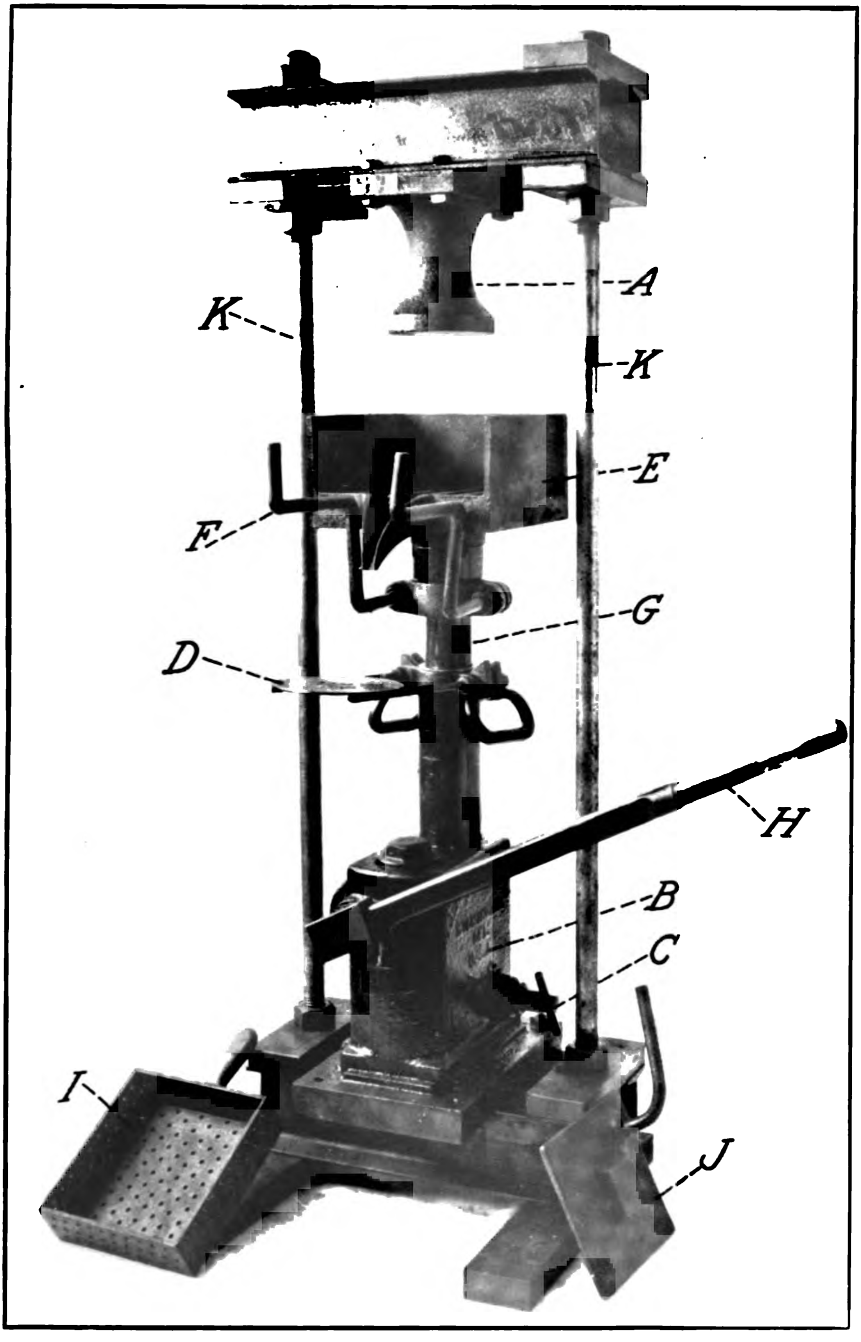
[1] Taken from report of E. E. Ewell, Fifty-sixth Congress, first session, Doc. No. 699, p. 147.



HYDRAULIC PRESS FOR BEET ANALYSIS.

A, Head block. *B*, Hydraulic jack with ram *G*. *C*, Release valve of jack. *D*, Stand for vessel to receive juice. *E*, Receiving box fastened to ram *G*. *H*, Handle for pumping up jack. *I*, *J*, Porous box and cover for beet sample.

HYDRAULIC PRESS.

In Plate I is shown a form of press that has been used successfully for beet pressing by this laboratory. It consists of a hydraulic jack *B* fastened to two pieces of I beam clamped together for the base. Fastened to these clamps are two upright steel rods *K*, and to them are fastened I beams with clamps to hold the head block *A*. Fitted to the ram *G* of the jack is an iron box with lip *E*, also the bent rods *F* and the platform *D*. To operate this press the porous cast-iron pan *I* is placed in the box *E*. A square of jute cloth is then placed in the pan, and the sample of beet cuttings poured into it. The edges of the cloth are folded over and the plate *J* is placed on top. A jar for receiving the juice is placed on the platform *D*, and by means of the handle *H* the jack is jumped up against the head block *A*. When the juice has stopped running, the release valve at *C* is opened and the ram quickly returns to place. The pan *I* can be slid out on the rods *F*, the press cake removed, and a new sample inserted. It is essential to have a pressure gauge on the chamber of the jack, since in analyzing beets by this method the same pressure should be maintained throughout.

DIRECT METHODS.

In the direct methods the per cent of sugar is determined directly on the beet. These methods may be classified according to the solvent used for extraction, namely, water or alcohol, and these may be again divided into extraction in the cold and with heat. So many slight modifications of these different methods are known and used that all of them can not be discussed; only statements of the representative methods can be given.

WATER METHODS.

COLD-WATER DIGESTION METHOD OF PELLET.

Weigh the normal amount of beet cuttings, 26 or 26.048 grams, and transfer to a large-mouth flask (*C*, frontispiece) with a mark at 200.6 cc.[1] Add 5 cc of basic lead acetate solution (see p. 13), shake, and add water up to the shoulder of the flask. Mix the contents by rotating in the hand, and allow to stand 25 minutes in order to expel the air bubbles. Beat down the collected froth with an ether spray and fill the flask to the mark with water. Then shake the contents vigorously, placing the hand over the mouth of the flask, filter, and polarize in a 200 or 400 mm tube after adding a drop of acetic acid.

For this method it is necessary to have the finest divided pulp possible and it is also advisable to have a pear-shaped flask instead of the ordinary round, ball-shaped one in general use, so that the entrained air bubbles may seek the surface and not remain on the sides of the vessel. This method has been much criticized because a finely divided pulp is necessary to prevent an imperfect extraction of the sugar, and also because ordinary shaking will not disengage all of the air bubbles from the pulp, thereby causing the use of a smaller quantity of water to fill the flask to the mark, and thus giving too high results for sugar in the beet.

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[1] Numerous experiments have shown that the marc of the normal weight of average beets occupies about 0.6 cc of space.

COLD-WATER DIGESTION METHOD OF SACHS LE DOCTE.

This is a modification of Pellet's method for the purpose of removing the error resulting from the entrained air. Weigh 26 grams of the fine beet pulp into a tin-lined copper vessel (*D*, frontispiece), add 5 cc of basic lead-acetate solution and 172 cc of water. Put the cover *E* in place and shake the whole vigorously, then allow it to stand for three minutes, remove the cover, filter, and polarize the solution in a 200 mm tube after adding a drop of acetic acid and double the reading, or polarize in a 400 mm tube, in which case the reading gives the per cent of sugar in the beet.

In this method it is assumed that beets contain 95 per cent of juice with an average specific gravity of 1.07. The volume of the juice contained in a normal weight would then be

$$\frac{26\times 0.95}{1.07}=23.08 \text{ cc}$$

and (200–23.08 cc) 176.92 cc of water would be necessary to complete the volume to 200 cc. A special pipette (*F*, frontispiece) has been designed to deliver this amount. A quarter turn of the stopcock opens the entrance *G* for the lead acetate, so that 5 cc can be measured, then another quarter turn opens the water entrance *H* to fill the burette to the overflow, and a half turn delivers the whole into the dish.

As in the preceding method, the beet pulp must be very fine, otherwise an imperfect extraction will occur. This method is also open to criticism in that all beets do not contain 95 per cent of juice and all beet juice does not possess a specific gravity of 1.07. It is rightly claimed, however, that the errors introduced by the use of these constants are so small that they will fall within the limits of accuracy of the readings.

HOT-WATER DIGESTION METHOD.

Weigh 52 grams of the beet cuttings and transfer them with water to a large-mouth flask (*C*, frontispiece) of 201.2 cc content; add from 5 to 10 cc of lead subacetate solution, fill the flask to the mark with hot water, and shake. Immerse the flask in a water bath at 80° C. and shake at intervals by rotating. Add water from time to time so that at the end of the heating, about 30 minutes, the water in the flask is a little above the mark. Remove the flask from the water bath and allow it to cool to standard temperature; add a sufficient quantity of concentrated acetic acid to make the solution very slightly acid in reaction (generally less than 0.5 cc is necessary) and a few drops of ether to break the foam; complete the volume. Mix thoroughly, filter, and polarize in a 200 mm tube.

With this method a coarser beet pulp can be used than for the cold-water methods, but over 30 minutes may be necessary for digestion, if very coarse cuttings are used. To obtain correct results, care must be exercised to make up to volume at the standard temperature of 20° C. (if the instrument and flasks are standardized at that temperature) and to digest the beet cuttings with as large a quantity of water as possible. Not over 5 cc in any case should be added during digestion and final cooling to complete the volume. Where smaller quantities of water are used during digestion and then a large quantity of water is added at the last to make to volume, the sugar has not become equally diffused and the results are too low. Differences of over 1 per cent in sugar content are often caused by lack of care in this particular.

HOT-WATER DIGESTION METHOD OF SACHS LE DOCTE.

The procedure in the Sachs Le Docte cold-water extraction method (p. 18) is modified as follows for hot digestion: The weighing and the vessels used are the same, also the quantities of lead subacetate and water are the same, namely, 177 cc. A special rubber disk cover (*I*, frontispiece) is provided for the digestion vessels. Put this in place and after shaking the vessel immerse it in a water bath kept at 80° C. for 30 minutes, or for 25 minutes if the temperature is 85° C. The temperature during extraction should not, however, exceed this figure. Remove the cups and immerse in cold water, bringing the temperature down to 20° C., shake, remove the covers, filter, and polarize, after adding a drop or so of acetic acid.

The chance of error due to contained air or unequal diffusion of the pulp is removed by this method. As in the former case the cuttings need not be so fine as with the cold-water extraction methods.

HERZFELD'S MODIFICATION OF THE SACHS LE DOCTE METHOD.

Instead of the tin-coated copper beakers used in the preceding method, Herzfeld uses an extraction vessel of nickel-plated sheet iron made as shown in figure 5. The vessel is round. He also uses small weighing glasses, holding 26 grams of material, which can be introduced with the beet cuttings into the extraction vessel. These watch glasses are filled to equal weight and numbered consecutively, as are also the extraction vessels. The procedure is as follows:

Weigh 26 grams of the beet pulp on a watch glass and transfer to the extraction vessel, then run in 177 cc of dilute basic lead-acetate solution (5 parts of basic lead-acetate solution, Brix 53.5, to 100 parts of water), shake and place a stopper which has been covered with tinfoil lightly in the opening. Submerge the whole in a water bath at 75° to 80° C. for 30 minutes, shaking intermittently. When all air has been expelled (generally after five minutes), tighten the stopper in the vessel. At the expiration of the time remove and cool. Take out the stopper after shaking thoroughly, filter, and polarize in a 400 mm tube, after addition of a drop of acetic acid to determine the per cent of sugar in the beet.

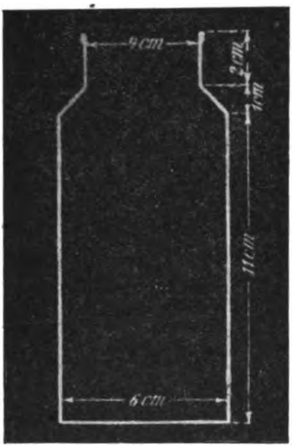

FIG. 5.—Herzfeld's metallic beaker.

This method does not require very fine pulp and is open to few chances of error. It, together with the Sachs Le Docte method, has a decided advantage over the other hot digestion method, in that there is little chance of a loss of sample by the container breaking. When working with glass flasks one is likely to ruin many determinations. For quick work and when many samples are to be run, large heating and cooling vessels can be used and the dishes taken from the one and placed immediately in the other to cool, without risk of losing the determination, thereby saving considerable time. This method has been adopted by the Society of the German Sugar Industry, and with a few modifications in apparatus, but not of procedure, by a number of other sugar associations of Europe.

ALCOHOL METHODS.

The methods using alcohol as the solvent may be divided into two classes—digestion and extraction methods. The former is again divided into hot and cold digestion methods.

COLD ALCOHOL DIGESTION METHOD.

Weigh 52 grams of the beet cuttings and transfer to a flask (having a capacity of 201.2 cc) with 90 per cent alcohol, add 4 cc of lead subacetate solution and shake, then add more 90 per cent alcohol with shaking to remove the air bubbles, complete the volume, allow to stand half an hour, and add alcohol if a decrease in volume is noted. Thoroughly shake the flask and filter, keeping the filter covered to prevent loss by evaporation. Polarize in a 200 mm tube.

With this method very fine pulp is necessary and the greatest care must be exercised to remove all of the air from the beet cuttings to insure an even digestion. For accurate readings the polarization should be made at the standard temperature of the instrument and flasks and allow of no evaporation. This method is but little used.

HOT ALCOHOL DIGESTION METHOD.

The same procedure is followed as in the preceding method up to the point of adding more alcohol. In this method add only enough 90 per cent alcohol to fill the flask three-fourths full. Then connect the flask with a return condenser, place in a water bath, and allow to boil for 20 minutes. Cool the flask and contents to the standard temperature and bring up to the mark with 90 per cent alcohol, shake and allow to stand for awhile, filter, and polarize.

This method does not require so fine a pulp as the former one and is not open to error from entrained air bubbles, but to obtain correct results it must be worked carefully to prevent evaporation and changes of temperature during polarization.

ALCOHOL EXTRACTION METHOD.

The alcohol extraction method has been recognized as the standard method for sugar determination and is the one with which other methods are compared, but its execution is difficult and the results are liable to error if the greatest care is not exercised. For the inexperienced chemist it is not a suitable method, but by familiarizing oneself with its details and difficulties, correct results can be obtained.

A Soxhlet extraction apparatus is necessary for this method. The usual form has been improved by opening the siphon tube with a short tube and cork as shown in the frontispiece, *K*. In this way one can test the progress of the extraction by withdrawing a sample without interfering with the work. The best procedure for this method is to weigh 26 grams of the beet pulp and transfer to a 100 cc flask with about 50 cc of 90 per cent alcohol and from 3 to 5 cc of basic lead-acetate solution. Connect a return condenser to the flask and place in a boiling water bath for from 10 to 15 minutes. Then pour the whole into the extractor, washing out the flask with fresh portions of 90 per cent alcohol. A return condenser is connected with the Soxhlet extractor and also a 100 cc flask, the latter by means of a cork. Add more 90 per cent alcohol until the siphon is started and the lower flask is about three-fourths full. Place the containing flask in a covered water bath held at a heat that will make the alcohol boil freely. Continue the extraction for from one to four hours, or until a test of the alcohol in the extractor gives no color with α-naphthol solution (see p. 22). Remove the flask and add 90 per cent alcohol to the mark after cooling to the standard temperature, shake and filter. Polarize in 200 mm tube.

Care must be exercised to prevent evaporation and changes of temperature and also to use only a minimum amount of basic lead acetate, generally nearer 3 cc than 5 cc, for clarification. By digesting the beet pulp with the alcohol before extraction, the time of extraction is greatly shortened, the pulp becomes thoroughly impregnated with the alcohol, and all air is removed, resulting in a good extraction of the whole material. If the pulp is fine and tends to clog the siphon, alcohol-washed cotton may be used as a plug in the extractor before adding the beet pulp, and a fine mesh screen may be placed over the pulp to keep the whole compact in the extractor.

To determine whether all of the sugar has been extracted or not the following qualitative test is used:

α-naphthol test for sugars.—Add four or five drops of a 20 per cent alcoholic α-naphthol solution to a few drops of the alcohol coming from the extractor and 2 cc of water contained in a small test tube. Shake well, tip the test tube, and allow from 2 to 5 cc of colorless concentrated sulphuric acid to flow down the side of tube; then hold the tube upright, and if sucrose is present a color varying from a faint to a deep violet will be noted at the junction of the two liquids. On shaking, the whole solution becomes a blue violet color. This test is suitable for the results required of it in this work, but it must be remembered that other sugars and substances besides sucrose give this color reaction.

DISCUSSION OF METHODS.

For a number of years the proper methods for determining sugar in the beet have been discussed by sugar chemists, especially as to the relative merits of alcohol and of water extraction. As a general rule German sugar chemists favored alcohol methods, while the French favored water methods. Within the last year a truce has been declared and it is acknowledged that for very accurate or control work the alcohol extraction method should be used, but for general work, one of the hot-water digestion methods will give good results and the analyst is less likely to introduce errors in the manipulation. With the average sample of beets, the two methods when carefully applied will yield duplicate results but in the case of very abnormal beets one method might give higher figures than another. The instantaneous methods (cold extraction) are only suitable with very fine pulp and the results can be depended upon only when they have been checked against a standard method. With these instantaneous methods as many samples of beets can be analyzed per day as by the indirect method, and it is claimed by many that the Sachs Le Docte hot digestion method or the Herzfeld modification will yield as many determinations in a given time as the indirect method, with the same amount of work, provided the laboratory is fitted for the work.

DETAILED ANALYTICAL DATA, 1905–1910.

Sugar-beet analyses, made in the Bureau of Chemistry.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
		Ounces.	*Per cent.*				
Alabama	Shelby □						
	Calera	13	13.0	77.6	Fair	7064	Aug. 20, 1909
Arizona	Yuma □						
	Yuma	16	14.6	77.2	Wilted	5626	Aug. 13, 1908
Arkansas	Cleveland □						
	Kingsland	8	11.4	80.3		4374	Aug. 25, 1906
	Craighead □						
	Jonesboro	14	7.40	57.6	Bad	7931	Oct. 17, 1910
	Dallas □						
	Ramsey	16	13.30	82.5	Good	7887	Oct. 3, 1910
	Do	20	13.00	80.8	do	7889	Do.
	Eaglette	14	12.15	79.7	do	7898	Oct. 8, 1910
	Faulkner □						
	Conway	5	15.2	78.8		5151	Nov. 15, 1907
	Pope □						
	Hector	16	13.0		Bad	7071	Aug. 21, 1909
California	Imperial □						
	Imperial	11	15.7	83.1	Good	7147	Oct. 3, 1909
	Modoc □						
	Alturas	40	13.75	76.3	do	7966	Oct. 24, 1910
	San Diego □						
	Lakeside	24	21.40	88.7	Fair	7888	Oct. 3, 1910
	Shasta □						
	Cassell	40	14.8	82.2	Good	7203	Oct. 19, 1909
	Siskiyou □						
	Macdoel	16	14.6	85.8	do	5721	Oct. 8, 1908
	Do	16	12.0	79.5	do	5722	Do.
	Do	10	8.0	63.5	Poor red	5725	Oct. 9, 1908
	Do	15	15.3	86.8	Good	5741	Oct. 12, 1908
	Do	4	15.5	85.6	Dry	5778	Oct. 17, 1908
	Do	16	18.1	91.8	Good	5792	Oct. 19, 1908
	Do	25	15.5	79.0	do	7128	Sept. 29, 1909
	Do	24	12.2	79.2	do	7142	Oct. 3, 1909
	Do	24	13.0	78.3	do	7145	Do.
	Do	8	12.7	76.8	do	7150	Do.
	Do	28	14.6	80.6	do	7175	Oct. 9, 1909
Colorado	Boulder □						
	Broomfield	20	10.00	70.6	do	8002	Nov. 1, 1910
	Eagle □						
	Avon	21	14.7	81.5		3760	Nov. 1, 1905
	Do	22	16.2	83.7		3761	Do.
	Bilkin	26	20.2	88.2		3769	Do.
	Eagle	26	15.9	85.0		3770	Do.
	Do	8	18.1	88.3		3771	Do.
	Do	40	20.9	88.7		3778	Nov. 8, 1905
	Avon	24	17.3	84.0		3802	Nov. 14, 1905
	Do	16	18.7	81.7		3811	Nov. 27, 1905
	Gypsum	32	14.6	78.6		3812	Do.
	Do	52	12.9	75.0		3816	Dec. 4, 1905
	Do	48	15.6	82.1		3817	Do.
	Do	20	22.6	89.3		5232	Dec. 9, 1907
	Do	17	18.1	84.1		5248	Dec. 23, 1907
	El Paso □						
	Ramah	16	16.3	83.2	Good	5700	Oct. 5, 1908
	Garfield □						
	Glenwood Springs	36	15.0	84.7		3701	Oct. 5, 1905
	Do	36	10.8	71.1		3702	Do.
	Do	44	15.4	75.5		3703	Do.
	Garfield	24	17.5	86.6		3765	Nov. 1, 1905
	Do	18	14.2	86.6		3766	Do.
	Do	24	11.9	78.8		3767	Do.
	Do	26	19.1	86.8		3768	Do.
	Do	56	17.5	83.5		3777	Nov. 18, 1905
	Do	24	19.2	83.5		3792	Nov. 14, 1905
	Kit Carson □						
	Seibert		10.7	66.5	Wilted	5660	Sept. 30, 1908
	Do	15	18.2	84.2	Good	5708	Oct. 6, 1908
	Burlington	19	10.7	72.3	do	5728	Oct. 9, 1908
	Seibert	22	15.0	87.2	do	5745	Oct. 12, 1908
	Do	16	16.4	88.6	Fair	5751	Oct. 13, 1908
	Do	24	9.8	71.2	do	5753	Do.
	Do	16	16.7	85.2	Good	7149	Oct. 3, 1909

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Colorado	Lincoln	*Ounces.*	*Percent.*				
	Limon	18	14.3	81.2	Good	7207	Oct. 19, 1909
	Do	24	9.0	58.8	do	7815	Aug. 6, 1910
	Otero						
	Rocky Ford	24	14.7	77.4	Very bad	7962	Oct. 21, 1910
	Pitkin						
	Aspen	14	17.0	77.8		3803	Nov. 20, 1905
	Routt						
	Steamboat Springs	14	14.1	77.0	Good	7923	Oct. 15, 1910
	Washington						
	Cope	4	14.8	78.7	Very small	5702	Oct. 5, 1908
	Do	15	17.5	82.3	Good	7196	Oct. 15, 1909
	Akron	48	14.0	75.2	do	7329	Nov. 8, 1909
	Do	32	17.4	78.4	Soft	7330	Do.
	Do	38	15.6	81.7	Good	7331	Do.
	Do	48	14.9	79.2	do	7332	Do.
	Do	32	16.2	81.8	do	7333	Do.
	Do	40	15.3	80.1	do	7334	Do.
	Do	45	16.1	82.1	do	7335	Do.
	Do	33	18.3	81.3	do	7336	Do.
	Do	29	20.9	86.4	Soft	7337	Do.
	Do	40	19.0	86.0	do	7338	Do.
	Do	56	18.7	81.3	Good	7339	Do.
	Do	29	18.6	83.4	do	7340	Do.
	Do	36	16.4	82.8	Good, soft	7341	Do.
	Do	28	17.7	80.8	Good	7342	Do.
	Do	37	18.4	84.0	Soft	7343	Do.
	Do	32	19.3	85.4	Good	7344	Do.
	Do	45	16.7	87.0	do	7345	Do.
	Do	26	19.4	85.8	do	7346	Do.
	Do	29	21.6	84.7	do	7347	Do.
	Do	40	19.7	86.3	do	7348	Do.
	Do	48	19.4	84.5		7349	Do.
	Weld						
	La Salle	24	19.9	85.6		3804	Nov. 21, 1905
	Carr	16	17.0	83.7	Fair	5774	Oct. 16, 1908
District of Columbia.	Washington.						
	Do	8	19.2	81.5	Soft	7350	Nov. 8, 1909
	Do	14	20.1	83.3	do	7351	Do.
	Do	28	18.5	75.5	do	7433	Dec. 2, 1909
	Do	22	21.6	78.7	do	7434	Do.
	Do	20	20.7	78.3	do	7436	Do.
	Do	28	13.1	60.1	Rotten, soft.	7437	Do.
	Do	56	17.2	70.1	do	7438	Do.
	Do	52	16.4	70.3	Bad	7439	Do.
	Do	36	17.2	71.1	Soft	7441	Do.
	Do	30	20.0	75.2	Fair	7442	Do.
Georgia	Chattooga						
	Summerville	10	6.2	58.3	Good	7857	Sept. 23, 1910
	Coweta						
	Newman	12	14.0	88.1	do	7903	Oct. 10, 1910
	Floyd						
	Rome	14	8.9	71.6	Good	7176	Oct. 9, 1909
	Greene						
	Penfield	48	7.8	66.6	Soft	5736	Oct. 10, 1908
	Do	17	12.3	80.9	Fair	5735	Do.
Idaho	Blaine						
	Picabo	10	16.5	84.1	Good	8017	Nov. 4, 1910
	Twin Falls						
	Twin Falls	24	20.1	89.5		4381	Oct. 10, 1906
	Do	24	17.7	82.9	Good	8091	Nov. 19, 1910
Illinois	Bond						
	Greenville	20	11.2	74.7	do	8044	Nov. 14, 1910
	Bureau						
	Princeton	48	14.9	76.4	do	7223	Oct. 21, 1909
	Do	21	12.1	76.6	do	7234	Oct. 23, 1909
	Do	20	12.8	77.4	do	7245	Oct. 25, 1909
	Do	48	11.7	72.6	do	7272	Oct. 28, 1909
	Do	32	12.8	76.6	do	7275	Do.
	Do	16	10.8	73.9	do	7290	Nov. 1, 1909
	Do	21	14.9	79.7	do	7294	Do.
	Do	60	10.5	73.2	do	8025	Nov. 8, 1910
	Cook						
	Blue Island	12	19.8	88.0	Fair	5856	Nov. 2, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
		Ounces.	*Per cent.*				
Illinois	Henry						
	Geneseo	32	12.9	80.1		3753	Oct. 31, 1905
	Do	20	10.5	70.2		3754	Do.
	Do	16	12.1	77.7		3755	Do.
	Do	24	11.1	76.1		3756	Do.
	Do	24	12.1	71.6		3757	Do.
	Do	8	14.7	84.0		3758	Do.
	Do	16	11.7	73.3		3759	Do.
	Do	22	13.7	74.6	Withered	4408	Nov. 26, 1906
	Do	16	15.2	78.4	do	4409	Do.
	Do	20	18.3	85.9	do	4410	Do.
	Do	22	16.6	82.6		4413	Do.
	Do	18	17.2	81.2		4414	Do.
	Do	16	19.0			4415	Do.
	Do	16	17.4			4416	Do.
	Do	14	18.9			4417	Do.
	Do	10	20.0			4418	Do.
	Do	10	17.7			4419	Do.
	Do	18	16.2	81.4		4420	Do.
	Do	14	14.7	76.6		4421	Do.
	Do	24	18.4	81.3	Withered	4427	Nov. 27, 1906
	Do	16	18.9	82.9	do	4428	Do.
	Do	38	16.9	81.7	do	4429	Do.
	Do	18	15.9	82.4	do	4430	Do.
	Do	38	13.4	80.7	do	4431	Do.
	Do	16	16.5	81.7	do	4432	Do.
	Do	16	17.8			4434	Do.
	Do	28	16.6	85.1	do	4435	Do.
	Do	16	17.2	86.0	do	4436	Do.
	Do	24	19.1	86.3	do	4437	Do.
	Do	16	17.2	83.0	do	4438	Do.
	Do	20	16.4	80.0	do	4439	Do.
	Do	14	16.7	86.6	do	4440	Do.
	Do	18	14.8	76.3	do	4441	Do.
	Lake						
	Waukegan	21	13.2	80.0	do	4442	Nov. 31, 1906
	Union						
	Reynoldsville	37	8.1	64.8	Good	7224	Oct. 21, 1909
	Woodford						
	Eureka	44	8.9	66.6	Withered	8007	Nov. 2, 1910
Indiana	Huntington						
	Warren	24	12.4	76.8	Good	7173	Oct. 8, 1909
	Newton						
	Goodland	36	13.8	79.7	do	8026	Nov. 9, 1910
	Ripley						
	Versailles	48	9.5	70.0	do	7970	Oct. 27, 1910
	Starke						
	North Judson	16	16.0	85.1	do	7129	Sept. 29, 1909
Iowa	Allamakee						
	Harpers Ferry	20	11.5	74.0	do	7146	Oct. 3, 1909
	Do	10	14.7	81.6	do	7228	Oct. 21, 1909
	Fayette						
	Clermont	39	9.9	70.7	Good-large	7095	Sept. 20, 1909
	Do	52	13.0	78.3	do	7151	Oct. 3, 1909
	West Union	16	13.0	79.1	Good	7235	Oct. 23, 1909
	Do	16	10.4	74.3	do	7246	Oct. 25, 1909
	Clermont	50	12.4	76.1	Good-large	7289	Oct. 30, 1909
	Jefferson						
	Fairfield	32	12.1	78.6		3737	Oct. 27, 1905
	Lyon						
	Rock Rapids	5	19.0	90.4	Fair	5719	Oct. 8, 1908
	Palo Alto						
	Cylinder	21	10.8	69.7		3725	Oct. 27, 1905
	Do	21	15.2	81.3		3726	Do
	Emmetsburg	30	10.2	73.4		3727	Do.
	Do	20	13.1	83.2		3728	Do.
	Do	42	10.3	74.9		3729	Do.
	Do	17	9.7	70.9		3730	Do.
	Do	17	15.3	84.3		3731	Do.
	Do	18	12.3	78.0		3732	Do.
	Do	32	11.7	79.0		3733	Do.
	Do	20	9.5	71.8		3734	Do.
	Do	40	10.7	74.3		3735	Do.
	Osgood	12	12.2	74.2		3738	Do.
	Rodman	26	9.8	69.6		3739	Do.
	Polk						
	Runnells	15	13.3	74.6		3779	Nov. 9, 1905

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Iowa	Winneshiek	*Ounces.*	*Per cent.*				
	Calmar	24	12.8	73.5	Good	7218	Oct. 21, 1909
	Decorah	48	8.2	63.5	do	7319	Nov. 4, 1909
	Ridgeway	28	12.8	81.0	do	7328	Nov. 8, 1909
	Woodbury						
	Fallow	24	13.7	79.2		3736	Oct. 27, 1905
	Sioux City	28	13.9	78.5		4893	Oct. 24, 1907
	Do	32	14.0	80.9		4894	Do.
	Do	16	15.2	81.3		4895	Do.
	Do	28	16.8	82.4		4899	Do.
	Do	32	14.6	80.7		4900	Do.
	Do	28	14.4	80.0		4901	Do.
	Do	28	15.6	78.8		4902	Do.
	Do	24	14.7	82.6		4903	Do.
Kansas	Chase						
	Elmdale	26	8.7	67.4	Withered	5674	Oct. 1, 1908
	Chautauqua						
	Chautauqua	24	14.6	80.4		4867	Oct. 10, 1907
	Do	24	14.0	80.5		4869	Do.
	Do	12	11.7	76.5		4890	Oct. 22, 1907
	Do	23	11.1	70.6	Slightly wilted.	5030	Nov. 7, 1907
	Do	12	8.6	58.9	Wilted	5031	Do.
	Do	16	9.5	63.3	do	5032	Do.
	Do	33	9.7	69.8	do	5033	Do.
	Do	19	12.6	77.8	do	5034	Do.
	Do	11	13.5	82.3	do	5035	Do.
	Peru	14	7.7	64.7	Withered	5036	Do.
	Chautauqua	9	11.8	71.7		5152	Nov. 15, 1907
	Finney						
	Garden City	32	15.0	80.2	Fair	5935	Nov. 6, 1908
	Kearny						
	Deerfield		14.7	82.1	do	5666	Oct. 1, 1908
	Do		9.2	71.9	do	5667	Do.
	Do		10.0	72.5	do	5669	Do.
	Marion						
	Florence	10	12.4	70.4	Good	8024	Nov. 8, 1910
	Rice						
	Sterling	26	10.7	76.9	do	5790	Oct. 19, 1908
	Russell						
	Russell	9	17.9	74.0	Very bad	7967	Oct. 24, 1910
	Do	2	19.3	91.2	Good (dirty)	7997	Oct. 31, 1910
	Wichita						
	Leoti	24	16.1	82.1	Good	7425	Nov. 29, 1909
Louisiana	Caddo						
	Shreveport	5	9.5	70.8	do	7863	Sept. 27, 1910
Maryland	Frederick						
	Frederick	21	17.4	79.8	Wilted	3823	Dec. 16, 1905
	Garrett						
	Garrett	6	17.6	83.1	Good	8031	Nov. 9, 1910
	Do	22	17.2	87.6	do	8032	Do.
	Do	8	13.5	80.0	do	8034	Do.
	Do	3	10.4	83.1	do	8035	Do.
	Do	17	17.6	90.7	do	8095	Do.
	Do	32	14.8	81.1	do	7933	Oct. 17, 1910
	Mountain Lake Park.	11	16.6	83.5	do	8036	Nov. 9, 1910
	Montgomery						
	Chevy Chase	19	13.9	77.6	do	7938	Oct. 18, 1910
Michigan	Kalkaska						
	South Boardman	19	16.4	85.4	Good	7354	Nov. 10, 1909
	Gratiot						
	Ithaca	16	19.3	83.5		4447	Nov. 2, 1906
	Do	16	20.0	88.2		4448	Do.
	Do	16	20.6	85.1		4449	Do.
	Do	16	22.2	89.1		4450	Do.
	Do	14	19.3			4451	Do.
	Do	18	18.1	84.7		4473	Nov. 19, 1906
	Do	26	16.6	83.4	Slightly moldy.	4491	Dec. 15, 1906
	Do	22	16.8	85.7	Very moldy.	4492	Do.
	Do	24	16.4	82.4	Good	4493	Do.
	Do	16	16.6	79.4	Moldy, dry	4494	Do.
	Do	22	15.2	82.2	Dry	4495	Do.
	Do	26	16.5	81.7	Shriveled and moldy.	4496	Do.
	Do	28	14.5	78.8	Good	4497	Do.
	Do	29	13.9	76.8	do	4498	Do.

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
		Ounces.	*Per cent.*				
Michigan	Gratiot	29	16.0	82.0	Good	4499	Dec. 12, 1906
	Ithaca	23	17.3	81.2	do	4500	Do.
	Do	25	16.1	81.7	do	4501	Dec. 15, 1906
	Lenawee						
	Blissfield	10	18.3	83.9		3782	Nov. 14, 1905
	Do	10	18.2	82.7		3783	Do.
	Do	8	18.8	82.5		3784	Do.
	Do	10	19.1	83.8		3785	Do.
	Do	16	19.8	83.2		3786	Do.
	Do	10	19.1	84.7		3787	Do.
	Do	16	18.4	82.5		3788	Do.
	Do	16	17.8	81.3		3789	Do.
	Do	10	18.5	82.6		3790	Do.
	Do	10	17.7	84.7		3791	Do.
	Do	24	9.8	70.3		3795	Nov. 16, 1905
	Do	12	13.1	73.8		3796	Do.
	Do	20	15.1	80.5		3797	Do.
	Do	24	8.4	67.2		3799	Do.
	Monroe						
	Dundee	12	20.9	92.5		3821	Dec. 9, 1905
	Do	20	17.7	87.6		3822	Do.
	St. Clair						
	Marine City	12	18.9	86.7		3772	Nov. 6, 1905
	Do	24	19.0	89.6		3773	Do.
	Do	16	18.2	86.3		3774	Nov. 4, 1905
	Do	16	20.0	86.6		3775	Do.
	Do	12	15.5	81.6		4424	Nov. 9, 1906
	Do	17	17.1	85.6		4480	Nov. 23, 1906
	Do	21	15.4	82.7		4481	Do.
	Tuscola						
	Caro	24	17.1	86.5		4377	Sept. 29, 1906
	Kingston	18	16.3	80.1		4388	Oct. 20, 1906
	Caro	16	14.7	80.6		4389	Oct. 30, 1906
	Kingston	24	16.0	82.5		4391	Oct. 27, 1906
	Do	22	22.2	88.5		4392	Do.
	Do	28	16.9	84.9		4394	Do.
	Colling	16	17.2	86.5	Good	7119	Sept. 28, 1909
	Washtenaw						
	Manchester	16	15.1	85.8		3815	Dec. 1, 1905
	Ypsilanti	34	13.5	81.0		4382	Oct. 11, 1906
	Milan	32	14.4	82.1		4484	Nov. 26, 1906
	Do	26	14.1	81.6		4485	Do.
	Do	15	14.8	83.6		4894	Oct. 24, 1907
	Do	22	18.0	88.7		4939	Nov. 5, 1907
Minnesota	Beltrami						
	Bemidji	9	9.8	66.6	Good	7994	Oct. 29, 1910
Mississippi	Adams						
	Natchez	32	1.3	17.5	do	7072	Aug. 23, 1909
	Do	26	6.4	43.8	Bad, rotten	7073	Do.
	Clay						
	West Point		13.0	78.3		5658	Sept. 29, 1908
	Lincoln						
	Brookhaven	10	11.3	76.4		7092	Sept. 20, 1909
	Do	12	12.0	75.9	Good	7114	Sept. 27, 1909
	Do	6	11.4	76.1	Poor, soft	7130	Sept. 30, 1909
	Do	10	10.9	74.1	Good	7193	Oct. 14, 1909
	Do	20	9.7	72.7	do	7895	Oct. 7, 1910
Missouri	Barton						
	Lamar	2	9.7	70.2	Poor, small	7108	Sept. 21, 1909
	Carter						
	Hunter	11	7.0	63.6	Good	7932	Oct. 17, 1910
	Hickory						
	Quincy	20	8.9	62.6	Poor, soft	7131	Sept. 30, 1909
	Do	19	10.0	69.3	Fair	7232	Oct. 22, 1909
	Howell						
	Willow Springs	8	14.0	84.3	Good	5791	Oct. 19, 1908
	Jasper						
	Jasper	13	12.0	77.3	do	7860	Sept. 26, 1910
	McDonald						
	Powell	9	8.2	65.6	do	7918	Oct. 11, 1910
	Newton						
	Seneca	6	12.1	75.0	Small	7177	Oct. 9, 1909
	Texas						
	Houston	6	9.8	72.9	Good	7891	Oct. 8, 1910
	No county.						
	St. Louis	32	14.0	86.2		6011	Nov. 14, 1908
	Washington						
	Mineral Point	5	14.3	84.9	Fair, small	7120	Sept. 28, 1909
	Wayne						
	Gads Hill		10.3	68.7		5657	Sept. 28, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Montana	Fergus	*Ounces.*	*Percent.*				
	Moore	22	16.6	84.8	Good	8043	Nov. 14, 1910
	Lewis and Clark						
	Augusta	24	16.7	76.8	do	7321	Nov. 4, 1909
	Yellowstone						
	Billings	5	16.9	87.4	do	7899	Oct. 8, 1910
Nebraska	Boyd						
	Butte	18	9.2	67.2	do	5732	Oct. 9, 1908
	Johnson						
	Cook		10.1	69.2	Fair	5671	Oct. 1, 1908
	Lancaster						
	Havelock	32	12.0	71.4	Good	7326	Nov. 8, 1909
	Lincoln						
	North Platte	21	9.5	69.8	Fair	5951	Nov. 9, 1908
	Do	18	9.9	69.2	do	5952	Do.
	Do	17	9.2	66.1	do	5953	Do.
	Scotts Bluff						
	Morrill	21	15.9	84.4	Good	7293	Nov. 1, 1909
	Do	24	18.7	85.0	Fair	7424	Nov. 29, 1909
	Do	17	16.3	82.5	Good	7426	Do.
Nevada	Churchill						
	Fallon	24	15.5	91.7	do	5809	Oct. 23, 1908
	Hazen	16	18.3	87.1	do	5949	Nov. 7, 1908
	Fallon	40	17.9	84.4	Fair	5992	Nov. 10, 1908
	Do	21	15.0	82.8	Good	6004	Nov. 12, 1908
	Do	9	17.4	83.2	Fair	6022	Nov. 17, 1908
	Do	24	16.4	83.2	Good	7148	Oct. 3, 1909
	Do	11	16.1	83.6	do	7188	Oct. 12, 1909
	Do	24	16.7	81.0	do	7277	Oct. 29, 1909
	Do	24	16.7	84.2	do	7278	Do.
	Do	19	19.5	89.0	do	7279	Do.
	Do	21	18.9	87.5	do	7280	Do.
	Do	17	20.3	91.4	do	7281	Do.
	Do	20	19.0	90.0	do	7282	Do.
	Do	27	21.0	92.5	do	7283	Do.
	Do	20	20.0	90.5	do	7284	Do.
	Do	24	19.6	89.5	do	7285	Do.
	Do	24	19.6	89.5	do	7286	Do.
	Hazen	48	16.6	82.6	do	7394	Nov. 19, 1909
New Jersey	Hudson						
	Bayonne	16	8.3	63.5	Fair	7091	Sept. 20, 1909
New Mexico	Chaves						
	Roswell	13	19.5	87.3		4619	Jan. 16, 1907
	Do	10	19.2	84.2		4620	Do.
	Do	10	20.4	86.4		4621	Do.
	Do	13	17.7	81.6		4622	Do.
	Colfax						
	Cimarron	28	17.5	80.4	Wilted	5207	Nov. 29, 1907
	Do	18	16.1	84.1	Fair	5090	Oct. 3, 1908
	Do	28	16.0	85.1	Good	5703	Oct. 5, 1908
	Curry						
	Texico	25	19.7	92.1	do	5946	Nov. 7, 1907
	St. Vrain	28	19.1	86.0	Fair	5948	Do.
	Eddy						
	Artesia	17	9.5	75.4	Red, soft	5795	Oct. 19, 1908
	Lincoln						
	Carrizozo	15	18.5	87.2		5255	Dec. 30, 1907
	Mora						
	La Cueva	16	19.7	84.9		5048	Nov. 8, 1907
	Do	16	18.2	80.9		5049	Do.
	Do	16	22.3	86.9		5238	Dec. 13, 1907
	Do	24	21.7	86.9		5239	Do.
	Do	19	17.0	78.7	Good	6033	Nov. 20, 1908
	Do	24	18.8	84.6	Fair	6082	Dec. 28, 1908
	Levy	18	12.0	73.9	Good	7187	Oct. 12, 1909
	Do	16	17.5	86.9	do	7323	Nov. 4, 1909
	Wagon Mound	30	16.6	82.5	do	7353	Nov. 9, 1909
	Quay						
	Nara Visa	6	10.7			6042	Nov. 23, 1908
	Roosevelt						
	Portales	18	15.2	82.3		4386	Oct. 12, 1906
	Claudell	7	20.9	88.6	Fair	5779	Oct. 17, 1908
	Upton	9	18.5	88.5	Poor	5815	Oct. 26, 1908
	Elida	17	19.4	95.1	Fair	5838	Oct. 27, 1908
	Portales	12	10.9	72.6	Good	7153	Oct. 5, 1909

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
New Mexico....	San Miguel	*Ounces.*	*Percent.*				
	East Las Vegas...	18	15.7	78.5	....	4908	Oct. 28, 1907
	Do...........	36	18.2	79.8	....	5058	Nov. 8, 1907
	Las Vegas.........	12	20.6	90.4	....	5079	Nov. 11, 1907
	Do...........	60	18.3	78.5	....	5080	Do.
	Rociada...........	16	18.7	81.3	....	5081	Do.
	San Jose..........	46	13.6	70.5	....	5082	Do.
	Las Vegas.........	10	20.8	88.8	....	5085	Nov. 12, 1907
	East Las Vegas...	22	18.1	80.4	....	5149	Nov. 14, 1907
	Do...........	15	22.2	90.2	....	5206	Nov. 25, 1907
	Las Vegas.........	8	22.1	....	Withered...	5240	Dec. 13, 1907
	Do...........	4	11.9	76.0	Withered, small.	5241	Do.
	East Las Vegas...	16	14.3	85.6	Fair.........	6052	Nov. 24, 1908
	Los Alamos.......	22	10.0	80.6	Poor.........	6056	Do.
	East Las Vegas...	32	13.6	77.3	Good........	7248	Oct. 26, 1909
	Las Vegas.........	56	14.8	77.3	.....do.......	7395	Nov. 19, 1909
	Do...........	17	16.8	77.0	Withered...	8156	Dec. 8, 1910
	Santa Fe						
	Otto...............	24	16.7	83.5	Fair.........	5688	Oct. 3, 1908
	Do...........	9	16.9	86.2	Small.......	5695	Oct. 5, 1908
	Do...........	34	17.1	86.3	Fair.........	5698	Do.
	Stanley...........	32	16.1	83.0	Good........	5699	Do.
	Otto...............	11	15.6	83.9	.....do.......	5701	Do.
	Stanley...........	15	15.7	86.7	.....do.......	5704	Do.
	Otto...............	17	16.5	89.2	.....do.......	5706	Oct. 6, 1908
	Hyer..............	35	16.0	84.6	.....do.......	5707	Do.
	Otto...............	13	13.8	80.0	Fair.........	5709	Do.
	Hyer..............	12	19.5	85.9	Good........	5713	Do.
	Otto...............	13	17.4	87.8	.....do.......	5720	Oct. 8, 1908
	Do...........	30	15.7	85.3	.....do.......	5726	Oct. 9, 1908
	Stanley...........	11	14.2	82.5	.....do.......	5730	Do.
	Do...........	13	18.2	91.0	Fair.........	5733	Do.
	Otto...............	52	10.5	71.9	Good........	5752	Oct. 12, 1908
	Stanley...........	19	17.0	85.0	Fair.........	5783	Oct. 17, 1908
	Do...........	16	21.5	95.1	.....do.......	5784	Do.
	Do...........	27	15.9	91.0	Good........	5800	Oct. 20, 1908
	Otto...............	35	11.9	84.3	.....do.......	5808	Oct. 23, 1908
	Stanley...........	15	21.9	....	....	5934	Nov. 6, 1908
	Sierra						
	Arrey.............	24	12.9	72.5	Soft.........	7231	Oct. 22, 1909
	Taos						
	Taos..............	22	17.0	73.8	Withered...	4464	Nov. 14, 1906
	Torrance						
	Moriarty..........	28	17.3	84.0	....	5675	Oct. 2, 1908
	Do...........	19	17.4	80.2	Withered...	5676	Do.
	Do...........	18	18.7	85.0	Fair.........	5689	Oct. 3, 1908
	McIntosh..........	24	15.7	86.2	Good........	5696	Oct. 5, 1908
	Moriarty..........	24	17.6	85.4	.....do.......	5697	Do.
	Do...........	12	16.9	84.5	.....do.......	5710	Oct. 6, 1908
	Do...........	11	18.5	81.0	.....do	5727	Oct. 9, 1908
	Do	10	17.3	88.3	.....do.......	5731	Do.
	Do...........	12	16.0	94.7	.....do.......	5742	Oct. 12, 1908
	Do...........	18	16.5	83.4	Fair.........	5746	Do.
	Estancia..........	20	11.0	72.6	.....do.......	5747	Do.
	Do...........	33	13.2	81.6	Good........	5749	Do.
	Moriarty..........	20	16.0	82.8	.....do.......	5750	Oct. 13, 1908
	Do...........	17	15.0	80.2	.....do.......	5754	Oct. 12, 1908
	Do...........	16	19.8	73.0	Fair.........	5772	Oct. 15, 1908
	Do...........	29	13.2	83.5	Good........	5793	Oct. 19, 1908
	Do...........	64	16.5	80.4	.....do.......	5796	Do.
	Do...........	4	13.5	85.4	Very small..	5812	Oct. 24, 1908
	Do...........	24	16.9	78.2	Fair.........	5901	Nov. 4, 1908
	Do...........	24	15.6	80.0	.....do.......	5903	Do.
	Union						
	Amistad..........	4	22.0	93.6	Very small..	5839	Oct. 28, 1908
	Clayton...........	12	17.0	86.2	Good........	7195	Oct. 15, 1909
	Do...........	16	16.3	86.2	.....do.......	7202	Oct. 19, 1909
	Do...........	9	14.5	75.5	.....do	7221	Oct. 21, 1909
New York......	Erie						
	Buffalo............	12	13.3	80.0	.....do.......	8008	Nov. 2, 1910
	Kings						
	Brooklyn..........	8	15.7	86.9	.....do.......	7862	Sept. 26, 1910

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
North Carolina	Ashe	*Ounces.*	*Percent.*				
	River Side	24	15.0	80.2	Good	7322	Nov. 4, 1909
	Beaufort						
	Washington	16	16.4	87.7	Wilted	4864	Oct. 10, 1907
	Do	16	14.0	83.3	do	4865	Do.
	Aurora	7	9.5	76.5		4885	Oct. 15, 1907
	Washington	36	11.6	75.3	Good	5620	July 31, 1908
	Do	16	7.8	70.0	Small	5655	Sept. 26, 1908
	Buncombe						
	Asheville	13	16.2	84.8		4936	Oct. 30, 1907
	Davie						
	Advance	5	11.0	78.5	Poor, small	7107	Sept. 21, 1909
	Guilford						
	Julian	32	11.8	82.5		4879	Oct. 14, 1907
	Haywood						
	Canton	24	14.2	82.7	Good	7974	Oct. 28, 1910
	Moore						
	Carthage	8	15.1	85.1	do	7144	Oct. 3, 1909
	Robeson						
	Maxton	16	12.5	83.3	Wilted	4866	Oct. 10, 1907
	Do	18	9.6	68.1		4868	Do.
	Watauga						
	Sands	18	17.6	86.5	Good	7204	Oct. 19, 1909
North Dakota	Barnes						
	Valley City	52	14.8	76.7	do	7992	Oct. 29, 1910
	Cavalier						
	Sarles	7	14.8	80.8	do	8000	Nov. 1, 1910
	Sargent						
	Cogswell	28	9.0	67.0	do	7891	Sept. 30, 1910
	Richland						
	Great Bend	28	11.4	68.8		3723	Oct. 18, 1905
	Do	44	11.5	68.2		3724	Do.
	Do	24	11.3	73.9		3749	Oct. 31, 1905
	Do	32	12.6	67.2		3750	Do.
	Do	48	14.7	78.4		3751	Do.
	Do	32	18.9	86.7		3752	Do.
	Walsh						
	Grafton	10	15.7	83.0	Good	7995	Oct. 29, 1910
	Wells						
	Hurdsfield	11	18.0	82.2	Fair	5687	Oct. 3, 1908
	Do	12	14.3	80.3	Good	5794	Oct. 19, 1908
	Do	24	16.2	85.7	do	5799	Oct. 20, 1908
	Do	17	16.6	86.5	do	5816	Oct. 26, 1908
	Do	10	16.0	94.1	Fair	5842	Oct. 27, 1908
	Do	20	16.1	90.9	Hard	5931	Nov. 5, 1908
	Do	15	8.2	65.5	Poor	5954	Nov. 9, 1908
Ohio	Brown						
	Ripley	16	10.0	73.0	Fair	6072	Dec. 9, 1908
	Columbiana						
	East Liverpool	24	10.4	69.3	Good	7919	Oct. 12, 1910
	Guernsey						
	Birds Run	13	14.6	80.6	do	8014	Nov. 3, 1910
	Hocking						
	Logan	24	16.8	84.6	do	7154	Oct. 6, 1909
	Marion						
	Marion	10	15.9	85.8	Fair	7233	Oct. 23, 1909
	Ottawa						
	Oak Harbor	20	17.5	84.9		4490	Dec. 12, 1906
	Paulding						
	Paulding	35	17.9	82.6		4407	Oct. 27, 1906
	Do	37	16.0	78.8		4422	Nov. 9, 1906
	Do	36	16.8	78.9		4423	Do.
	Haviland	12	21.2	88.0		4905	Oct. 28, 1907
	Do	8	21.0	86.4		4906	Do.
	Do	16	19.4	89.8		4907	Do.
	Do	18	19.3	89.8		5050	Nov. 8, 1907
	Do	22	16.8	88.2		5051	Do.
	Do	18	17.9	90.4		5052	Do.
	Do	15	18.4	89.3		5053	Do.
	Do	14	19.8	90.8		5054	Do.
	Do	12	19.8	91.7		5055	Do.
	Do	13	17.2	88.2		5056	Do.
	Do	13	16.9	89.2		5057	Do.
	Broughton	9	21.0	81.6	Dry, wilted	5158	Nov. 20, 1907
	Haviland	16	21.7	82.2	Badly wilted	5160	Nov. 22, 1907
	Van Wert						
	Scott	8	19.4	87.4	Wilted	5159	Do.
	Do	20	17.0	85.6		5211	Dec. 2, 1907
	Williams						
	Bryan	8	16.9	83.3	Very dry	4617	Jan. 9, 1906

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Oklahoma	Beaver	*Ounces.*	*Percent.*				
	Floris	48	14.9	86.6	Fair	5849	Oct. 31, 1908
	Do	26	14.2	79.7	Good	6003	Nov. 12, 1908
	Do	20	15.1	79.1	Fair	6012	Nov. 14, 1908
	Do	34	15.5	81.5	do	6014	Do.
	Do	11	14.1	80.6	do	6019	Nov. 16, 1908
	Do	8	16.7	83.9	do	6035	Nov. 20, 1908
	Do	7	17.4	84.6	Good	7201	Oct. 19, 1909
	Do	10	15.0	74.2	do	7209	Do.
	Do	6	17.5	78.5	do	7392	Nov. 19, 1909
	Do	20	13.4	77.6	Fair	8160	Dec. 13, 1910
	Carter						
	Ardmore	24	12.9	91.9	Good	7327	Nov. 8, 1909
	Comanche						
	Chattanooga	13	11.0	68.7	do	7427	Nov. 29, 1909
	Harmon						
	Vinson	7	16.2	78.2	Fair	7109	Sept. 24, 1909
	Kiowa						
	Mountain Park	22	11.6	78.6	Good	7861	Sept. 26, 1910
	Swanson						
	Snyder	24	14.9	81.6	do	7836	Sept. 19, 1910
	Do	9	16.6	89.8	do	8020	Nov. 8, 1910
	McIntosh						
	Hoffman	15	12.7	91.3	Good, soft	7106	Sept. 21, 1909
	Grayson	10	5.8	50.0	do	7185	Oct. 12, 1909
	Muskogee						
	Braggs		11.5	76.1		5668	Oct. 1, 1908
	Do	19	10.1	72.7	Good	7094	Sept. 20, 1909
	Do	26	13.6	75.8	do	7113	Sept. 27, 1909
	Wybark	12	14.0	80.7	do	7115	Do.
	Braggs	16	10.9	71.2	Fair	7117	Sept. 28, 1909
	Do	32	6.2	50.8	do	7152	Oct. 5, 1909
	Council Hill	2	13.2	77.0	Fair, small	7184	Oct. 11, 1909
	Okfuskee						
	Castle	16	8.5	64.9	do	7229	Oct. 21, 1909
	Roger Mills						
	McArthur	26	11.9	74.4		4887	Oct. 18, 1907
	Grimes	4	16.5	83.5	Good	7199	Oct. 16, 1909
	Washita						
	Port	26	13.4	78.8		5150	Nov. 15, 1907
	Woods						
	Alva	20	20.8	81.1	Dry	5237	Dec. 11, 1907
Oregon	Crook						
	Tumalo	16	17.0	85.0	Very dry	3704	Oct. 12, 1905
	Do	16	13.0	76.9		3705	Oct. 16, 1905
	Klamath						
	Klamath Falls	32	21.9	92.0	Wilted	5037	Nov. 8, 1907
	Do	15	15.7	77.7		5083	Nov. 11, 1907
	Merrill	30	16.5	79.6		5210	Dec. 2, 1907
	Klamath Falls	16	20.6	90.7	Fair	5748	Oct. 12, 1908
	Do	10	16.5	86.8	do	5775	Oct. 17, 1908
	Do	24	16.3	81.0	do	6015	Nov. 14, 1908
	Do	37	18.3	76.0	Withered	8029	Nov. 9, 1910
	Merrill	18	14.5	76.0	Good	7896	Oct. 7, 1910
	Do	10	14.5	80.8	do	7897	Oct. 8, 1910
	Do	32	14.5	77.1	Bad	7969	Oct. 27, 1910
	Do	16	18.0	87.9	Good	8009	Nov. 3, 1910
	Do	16	18.8	87.8	do	8011	Do.
	Do	13	19.3	87.7	do	8012	Do.
	Do	13	18.3	88.8	do	8013	Do.
	Do	16	20.4	88.7	do	8015	Do.
	Do	26	13.4	74.7	do	8016	Do.
	Do	35	17.3	76.1	Withered	8027	Nov. 9, 1910
	Do	28	15.9	74.7	do	8028	Do.
	Do	12	18.5	88.0	Good	8041	Nov. 14, 1910
	Do	24	16.0	82.0	do	8042	Do.
	Do	20	18.5	88.3	do	8048	Nov. 19, 1910
	Lincoln						
	Elk City	32	12.8	67.9	Withered	4446	Nov. 30, 1906
Pennsylvania	Center						
	State College	12	14.9	81.4	Good	7181	Oct. 11, 1909
	Lancaster						
	Manheim		16.9	84.0	Fair	5665	Oct. 1, 1908
	Philadelphia						
	Philadelphia		10.3	71.5	Small	5661	Sept. 30, 1908
	Potter						
	Ulysses	3	16.1	79.7		5208	Dec. 2, 1907
	Do	18	17.5	91.6	Good	5717	Oct. 7, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
South Dakota..	Butte ◰	*Ounces*	*Percent.*				
	Vale	16	18.0	75.3	Pithy	4903	Oct. 24, 1907
	Stanley □						
	Philip	14	19.5	83.7	Very soft	7428	Nov. 30, 1909
Tennessee	Coffee □						
	Tullahoma	12	8.7	69.6	Fair	7096	Sept. 21, 1909
	Do	5	11.9	77.1	Good	7222	Oct. 21, 1909
	Do	17	8.7	66.4	do	7247	Oct. 25, 1909
	Do	22	13.8	76.7	Fair	7429	Nov. 30, 1909
	Dickson ⬒						
	Tennessee City	19	7.6	62.2	Good	7088	Sept. 16, 1909
	Franklin ⬓						
	Winchester	24	10.8	75.5	Fair	7172	Oct. 8, 1909
	Grainger ◳						
	Rutledge	21	13.6	81.7	Good	7236	Oct. 23, 1909
	Hamblen ◳						
	Morristown	16	10.1	73.7	do	7118	Sept. 28, 1909
	Do	12	10.9	76.2	Fair	7288	Oct. 30, 1909
	Hickman □						
	Vernon	21	8.0	61.6	Poor	7090	Sept. 20, 1909
	Do	20	9.3	73.2	Good	7093	Do.
	Do	7	10.2	71.3	do	7393	Nov. 19, 1909
	Do	40	6.9	60.0	do	7999	Oct. 31, 1910
	Humphreys □						
	McEwen	4	13.9	83.2	Fair	7089	Sept. 17, 1909
	Do	9	14.7	85.6	Good	7886	Oct. 4, 1910
	Warren □						
	McMinnville	22	5.9	47.2	Fair	7205	Oct. 19, 1909
Texas	Armstrong ◰						
	Goodnight	22	15.4	84.2	Good	7902	Oct. 8, 1910
	Brown □						
	Blanket	19	13.1	74.0	do	6038	Nov. 20, 1908
	Carson ◰						
	Groom	60	12.8	76.4		3706	Oct. 16, 1905
	Do	10	13.3	74.5		3707	Do.
	Do	56	12.8	71.5		3708	Do.
	Do	64	13.2	73.3		3710	Do.
	Do	35	14.0	80.5		5233	Dec. 10, 1907
	Cass ◳						
	Avinger	20	8.3	64.9	Good	8023	Nov. 8, 1910
	Castro ◰						
	Summerfield	6	15.9			6043	Nov. 23, 1908
	Comanche □						
	Comanche	18	13.0	77.4	Soft	6037	Nov. 20, 1908
	Dallam ◰						
	Dalhart	24	14.2	81.6	Small, very dry.	3712	Oct. 18, 1905
	Do	12	13.9	79.1	Very dry	3713	Do.
	Do	16	13.0	73.6	Dry	3714	Do.
	Do	12	14.0	79.3	do	3715	Do.
	Do	19	15.3	81.2		5028	Nov. 7, 1907
	Do	12	16.5	86.0	Good	6010	Nov. 13, 1908
	Texline	26	12.0	71.4	do	7182	Oct. 11, 1909
	Do	16	14.2	77.1	do	7183	Do.
	Do	16	12.5	70.5	do	7273	Oct. 28, 1909
	Do	16	15.4	79.6	do	7291	Nov. 1, 1909
	Dallas ◳						
	Dallas	16	3.0	35.7		5084	Nov. 11, 1907
	Donley ◰						
	Jericho	44	10.3	70.3		3709	Oct. 16, 1905
	Do	32	13.8	74.6		4444	Nov. 31, 1906
	Clarendon	31	15.5	77.4		4465	Nov. 14, 1906
	Do	25	12.6	73.3		5153	Nov. 19, 1907
	Erath ⬒						
	Stephenville	20	12.2	70.1	Fair	6039	Nov. 20, 1908
	Dublin	35	11.4	72.1	do	6040	Do.
	Fannin ◳						
	Honey Grove	20	17.8	82.9	do	7864	Sept. 27, 1910
	Do	12	14.2	80.8	Good	7872	Oct. 1, 1910
	Do	28	15.2	80.7	do	7873	Sept. 30, 1910
	Do	28	17.3	84.7	do	7874	Do.
	Do	16	13.4	70.5	do	7890	Oct. 3, 1910
	Do	13	11.6	75.5	do	7941	Oct. 19, 1910
	Gray ◰						
	McLean	16	10.0	70.2	Very dry	3721	Oct. 18, 1905
	Do	20	7.9	58.5	do	3722	Do.
	Do	40	11.9	77.8		4375	Sept. 25, 1906
	Do	40	16.3	82.9		4379	Oct. 8, 1906
	Do	26	15.3	74.7		4445	Nov. 31, 1906
	Do	16	14.2	83.5	Fair	5850	Oct. 31, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Texas	Hamilton	*Ounces.*	*Per cent.*				
	Hico	36	13.3	79.3	Good	8022	Nov. 8, 1910
	Hartley						
	Channing	7	10.7	72.3		3716	Oct. 18, 1905
	Do	20	13.6	78.6	Very dry	3717	Do.
	Do	12	7.5	60.0	do	3718	Do.
	Do	20	13.6	76.8	do	3719	Do.
	Do	20	13.0	73.0	do	3720	Do.
	Hartley	32	11.5	69.3	Withered	4395	Oct. 27, 1906
	Channing	33	17.9	79.4	do	4443	Nov. 31, 1906
	Do	30	14.3	72.9	do	4453	Nov. 5, 1906
	Hunt						
	Cash	11	12.2	77.2	Fair	6018	Nov. 16, 1908
	Jeff Davis						
	Valentine	16	18.0	86.5	do	6051	Nov. 24, 1908
	Kaufman						
	Terrell	10	10.6	66.1	do	7922	Oct. 14, 1910
	Oldham						
	Wildorado	16	16.5	83.3	do	6069	Dec. 5, 1908
	Parmer						
	Bovina	28	13.8	72.2		4387	Oct. 19, 1906
	Do	6	18.7	89.5	Good	7324	Nov. 4, 1909
	Polk						
	Onalaska	33	8.3	53.2	Fair	7040	July 26, 1909
	Potter						
	Amarillo	40	11.1	70.0		3741	Oct. 31, 1905
	Do	36	16.7	82.8		3742	Do.
	Do	36	17.0	82.1		3743	Do.
	Do	32	12.8	78.8		3744	Do.
	Do	24	11.3	76.0		3745	Do.
	Do	16	16.3	84.0		3746	Do.
	Do	28	16.0	83.1		3747	Do.
	Do	32	18.3	87.7		3748	Do.
	Do	16	11.2	75.2		4380	Oct. 8, 1906
	Do	21	16.7	81.8		4463	Nov. 13, 1906
	Do	27	15.4	73.2		4884	Oct. 15, 1907
	Do	7	16.5	80.5	Healthy	4937	Oct. 31, 1907
	Do	6	20.1	85.5	do	4938	Do.
	Do	27	13.1	76.7		5059	Nov. 9, 1907
	Do	19	14.4	81.8	Good	5994	Nov. 10, 1908
	Do	10	14.3	75.2	do	7356	Nov. 16, 1909
	Do	16	14.9	77.2	do	7357	Do.
	Do	16	15.6	78.7	do	7358	Do.
	Do	18	15.8	78.6	do	7359	Do.
	Do	16	15.3	79.2	do	7360	Do.
	Do	16	16.4	79.2	do	7361	Do.
	Do	18	15.5	79.0	do	7362	Do.
	Do	16	17.5	81.3	do	7363	Do.
	Do	16	17.9	83.2	do	7364	Do.
	Do	16	18.4	84.0	do	7365	Do.
	Do	10	18.2	78.1	do	7366	Do.
	Do	13	16.4	77.0	do	7367	Do.
	Do	13	17.0	79.5	do	7368	Do.
	Do	10	17.4	77.2	do	7369	Do.
	Do	10	18.1	79.4	do	7370	Do.
	Do	16	15.8	75.9	do	7371	Do.
	Do	10	17.4	80.2	do	7372	Do.
	Do	13	17.7	77.6	do	7373	Do.
	Do	16	16.7	77.7	do	7374	Do.
	Do	16	16.7	79.1	do	7375	Do.
	Randall						
	Canyon	25	15.7	89.2	do	5744	Oct. 12, 1908
	Reeves						
	Pecos	18	16.4	75.2	Fair	6077	Dec. 28, 1908
	Runnels						
	Ballinger	12	14.0	79.1	Fair	6055	Nov. 24, 1908
	Scurry						
	Hermleigh	32	9.9	75.6		5680	Oct. 2, 1908
	Do	48	13.1	82.3	Poor	5681	Do.
	Do	11	15.6	81.2	Dry	5777	Oct. 17, 1908
	Pyron	16	16.5	80.0	Fair	5987	Nov. 10, 1908
	Winston	32	14.2	79.3	do	6028	Nov. 17, 1908
	Sherman						
	Stratford	16	16.3	74.0	Wilted	4502	Dec. 28, 1906
	Do	11	16.0	74.3	do	4503	Do.
	Tarrant						
	Keller	5	5.0	51.0	Small, soggy.	5729	Oct. 9, 1908
	Fort Worth	13	10.4	69.8	Fair	6036	Nov. 20, 1908

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Texas	Van Zandt	*Ounces.*	*Per cent.*				
	Canton	16	9.0	79.6	Poor	5743	Oct. 12, 1908
	Victoria						
	Victoria	56	7.3	52.9	Wilted and moldy.	5603	July 1, 1908
	Wheeler						
	Ramsdell	16	16.5	88.2	Good	6025	Nov. 17, 1908
	Wilbarger						
	Vernon	32	10.9	79.0		4371	Sept. 24, 1906
	Do	32	10.2	73.9		4372	Do.
	Do	20	11.5	78.3		4373	Do.
	Do	16	13.4	74.6	Withered	4452	Nov. 3, 1906
Utah	Juab						
	Nephi	9	11.9	75.1	Good	7206	Oct. 19, 1909
	Do	9	13.6	80.8	do	7208	Do.
	Do	6	14.3	80.5	do	7214	Do.
	Do	8	15.1	80.6	do	7215	Do.
	Millard						
	Oasis	13	14.2	80.0	do	7929	Oct. 15, 1910
	Do	24	15.8	80.1	do	7940	Oct. 19, 1910
	Do	36	14.1	78.6	do	7971	Oct. 27, 1910
	Do	13	16.8	91.5	do	7996	Oct. 31, 1910
	Do	24	15.8	85.1	do	8006	Nov. 2, 1910
	Do	24	15.6	80.3	do	8019	Nov. 8, 1910
	Do	18	18.2	89.3	do	8053	Nov. 18, 1910
	Hinckley	16	16.6	84.0	do	7954	Oct. 20, 1910
	Do	22	15.3	80.7	do	7955	Do.
	Do	52	14.7	80.7	do	8004	Nov. 2, 1910
	Uinta						
	White Rocks	35	16.0	90.9	Fair	5857	Nov. 2, 1908
	Do	20	15.0	86.2	Good	7904	Oct. 10, 1910
Virginia	Alexandria						
	Arlington	16	19.0	93.1	Fair	6026	Nov. 17, 1908
	Do	16	15.4	79.0	do	6027	Do.
	Do	21	16.0	84.6	do	6045	Nov. 23, 1908
	Do	33	12.0	73.6	do	6046	Do.
	Do	27	10.6	69.8	do	6047	Do.
	Do	23	12.2	75.3	do	6048	Do.
	Do	20	13.9	80.3	do	6049	Do.
	Do	8	10.7	76.9	Good	8037	Nov. 10, 1910
	Augusta						
	Stuarts Draft	16	14.7	87.5	do	5813	Oct. 23, 1908
	Do	12	14.7	83.0	Fair	6016	Nov. 16, 1908
	Do	10	17.0	92.4	do	6017	Nov. 14, 1908
	Do	17	12.5	78.4	Good	7132	Oct. 1, 1909
	Do	11	12.9	79.9	Fair	7133	Do.
	Do	9	14.0	80.2	Good	7211	Oct. 19, 1909
	Do	13	16.0	86.4	do	7212	Do.
	Do	24	13.9	79.7	do	7213	Do.
	Botetourt						
	Troutville	22	12.2	86.5	do	5806	Oct. 22, 1908
	Do	19	14.4	86.7	Fair	5807	Do.
	Do	18	16.3	92.0	Good	5814	Oct. 23, 1908
	Do	20	13.9	81.8	do	6008	Nov. 13, 1908
	Clarke						
	Berryville	15	14.1	85.4	Fair	5932	Nov. 5, 1908
	Do	16	12.6	75.9	Good	7097	Sept. 21, 1909
	Do	16	12.7	78.2	Fair	7098	Do.
	Do	16	13.6	80.4	Good	7099	Do.
	Do	17	10.5	76.6	do	7100	Do.
	Do	12	14.1	76.0	Fair	7127	Sept. 29, 1909
	Do	17	17.0	85.0	Good	7197	Oct. 16, 1909
	Do	18	16.9	82.8	do	7198	Do.
	Do	18	15.0	79.8	do	7226	Oct. 21, 1909
	Do	16	16.3	87.0	do	7227	Do.
	Do	26	15.0	76.1	do	7295	Nov. 1, 1909
	Fauquier						
	Rectortown	15	15.0	88.2	Fair	6001	Nov. 12, 1908
	Loudoun						
	Round Hill	20	13.8	80.7	Good	5805	Oct. 22, 1908
	Bluemont	18	16.0	81.6	Fair	5962	Nov. 10, 1908
	Do	12	15.8	85.4	Good	5988	Do.
	Round Hill	8	14.4	81.3	Fair	5998	Nov. 11, 1908
	Do	20	15.7	87.7	Good	6002	Nov. 12, 1908
	Bluemont	30	15.4	81.8	do	6009	Nov. 13, 1908
	Montgomery						
	Blacksburg	12	18.4	88.9	Fair	6034	Nov. 20, 1908
	Childress	32	5.3	62.0	do	6041	Nov. 21, 1908

Sugar-beet anaylses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Virginia	Pulaski □	*Ounces.*	*Percent.*				
	Dublin	32	16.5	86.4	Fair	5781	Oct. 17, 1908
	Do	13	17.5	88.8	do	5782	Do.
	Pulaski	26	14.0	87.5	do	5843	Oct. 30, 1908
	Do	18	15.5	83.7	do	5845	Do.
	Dublin	12	17.5	95.1	Good	5950	Nov. 7, 1908
	Pulaski	10	13.4	84.8	do	7122	Sept. 28, 1909
	Do	18	13.7	85.0	do	7123	Do.
	Do	17	12.5	79.6	do	7125	Do.
	Do	16	14.2	81.8	do	7238	Oct. 23, 1909
	Do	11	16.6	85.8	do	7239	Do.
	Do	16	15.3	84.5	do	7240	Do.
	Do	21	14.0	82.8	do	7241	Do.
	Dublin	16	10.4	71.2	do	7101	Sept. 21, 1909
	Do	24	11.7	76.4	do	7102	Do.
	Do	10	12.0	76.4	Fair	7103	Do.
	Do	14	12.0	78.4	do	7104	Do.
	Do	13	14.2	76.7	do	7242	Oct. 25, 1909
	Do	19	14.6	80.0	Good	7243	Do.
	Do	20	14.6	79.2	do	7244	Do.
	Do	14	12.5	77.1	do	7271	Oct. 27, 1909
	Roanoke □						
	Roanoke	16	14.0	....	Fair—soft	7124	Sept. 28, 1909
	Do	18	11.1	74.3	Good	7143	Oct. 3, 1909
	Do	34	13.4	78.2	do	7230	Oct. 22, 1909
	Rockingham □						
	Grottoes	39	10.5	68.1	Fair	6013	Nov. 14, 1908
	Smyth □						
	Seven Mile Ford	30	14.6	89.2	do	5737	Oct. 10, 1908
	Marion	22	16.6	91.6	do	5738	Do.
	Seven Mile Ford	48	13.9	86.3	do	5844	Oct. 30, 1908
	Atkins	23	16.5	85.1	do	5900	Nov. 4, 1908
	Do	29	16.0	71.1	do	5902	Do.
	Marion	21	16.5	86.3	Good	5945	Nov. 7, 1908
	Do	32	11.8	77.6	do	5947	Do.
	Seven Mile Ford	28	15.9	81.5	do	5989	Nov. 10, 1908
	Do	35	15.4	80.7	Fair	5990	Do.
	Do	24	15.3	79.3	do	5991	Do.
	North Holston	13	15.3	85.7	Good	7121	Sept. 28, 1909
	Do	22	13.6	83.2	do	7126	Do.
	Chilhowie	17	12.5	81.1	Fair	7134	Oct. 1, 1909
	Do	5	12.0	77.2	Good	7135	Do.
	Marion	32	12.5	76.2	do	7136	Do.
	Seven Mile Ford	42	13.4	77.2	do	7138	Oct. 2, 1909
	Do	13	10.7	72.7	do	7139	Do.
	Do	49	8.3	62.7	do	7186	Oct. 12, 1909
	North Holston	10	14.8	81.5	do	7191	Oct. 13, 1909
	Do	12	14.5	80.5	do	7192	Do.
	Marion	40	13.8	81.4	do	7210	Oct. 19, 1909
	Seven Mile Ford	42	14.3	82.5	do	7237	Oct. 23, 1909
	Do	28	11.3	70.6	do	7276	Oct. 28, 1909
	Chilhowie	13	11.3	77.6	do	7965	Oct. 24, 1910
	Do	17	11.3	74.0	do	8045	Nov. 14, 1910
	Southampton □						
	Ivor	17	14.0	79.0	Fair	7105	Sept. 21, 1909
	Tazewell □						
	Burkes Garden	20	13.6	81.4	Good	6005	Nov. 12, 1908
	Tazewell	30	17.1	87.7	do	6023	Nov. 17, 1908
	North Tazewell	8	8.4	60.8	do	7180	Oct. 11, 1909
	Warren □						
	Linden	20	13.7	79.1	do	6000	Nov. 11, 1908
	Do	36	15.5	83.7	do	6029	Nov. 17, 1908
	Washington □						
	Abingdon	15	16.9	85.3	do	5928	Nov. 5, 1908
	Do	15	16.8	89.8	do	5929	Do.
	Do	15	16.4	87.2	do	5930	Do.
	Konnarock	24	13.2	81.9	do	7116	Sept. 27, 1909
	Abingdon	17	14.5	78.8	do	7140	Oct. 2, 1909
	Glade Spring	10	14.9	82.7	do	7141	Do.
	Konnarock	18	15.0	83.3	do	7189	Oct. 13, 1909
	Do	34	13.0	81.2	do	7190	Do.
	Abingdon	19	14.8	82.6	do	7194	Oct. 14, 1909
	Do	24	11.1	76.0	do	7930	Oct. 17, 1910
	Do	36	13.0	81.9	do	7956	Oct. 20, 1910
	Do	24	14.4	82.9	do	8005	Nov. 2, 1910

Sugar-beet analyses, made in the Bureau of Chemistry—Continued.

State.	County and town.	Analysis.			Condition of sample.	Serial number.	Date of analysis.
		Average weight.	Sugar in juice.	Purity.			
Virginia	Wythe	*Ounces.*	*Per cent.*				
	Rural Retreat	16	13.8	78.8	Good	4883	Oct. 14, 1907
	Do	16	17.0	83.7		5154	Nov. 20, 1907
	Do	10	14.6	88.0	Fair	5996	Nov. 11, 1908
	Do	11	14.7	89.1	do	5997	Do.
	Do	34	17.7	84.6	Good	6032	Nov. 19, 1908
	Do	31	16.3	84.0	do	6044	Nov. 23, 1908
	Wytheville	8	19.0	82.5	Soft	7423	Nov. 23, 1909
Washington	Kitsap						
	Manette	9	19.2	89.0	Wilted	8157	Dec. 10, 1910
West Virginia	Jefferson						
	Shenandoah Junction.	16	12.3	70.1	Good		
	Charlestown	16	12.2	74.8	do	7928	Oct. 15, 1910
	Do	48	13.6	79.5	do	7957	Oct. 21, 1910
	Do	16	12.6	71.3	do	7958	Do.
	Do	32	15.0	82.6	do	7960	Do.
	Do	20	12.7	77.4	do	8018	Nov. 5, 1910
	Shenandoah Junction.	20	16.2	78.8	do	7968	Oct. 26, 1910
	Do	16	13.8	80.0	do	7972	Oct. 27, 1910
	Do	20	20.3	78.2	Bad	8003	Nov. 2, 1910
	Do	36	14.6	80.4	Good	8010	Nov. 3, 1910
	Kearneysville	20	13.2	81.1	do	7964	Oct. 24, 1910
	Do	16	14.7	77.3	do	8038	Nov. 10, 1910
	Do	6	17.7	84.0	do	8092	Nov. 23, 1910
	Summit Point	10	7.3	59.5	do	8021	Nov. 8, 1910
	Preston						
	Reedsville	21	18.3	86.9	do	8039	Nov. 10, 1910
	Roane						
	Bloomington	20	6.9	63.0	do	8001	Nov. 1, 1910
	Summers						
	Lowell	32	11.8	79.3	do	7993	Oct. 29, 1910
Wyoming	Carbon						
	Baggs	22	14.8	79.4	do	7892	Oct. 18, 1910
	Crook						
	Forest	32	11.5	74.2	do	7274	Oct. 28, 1909
	Laramie						
	Cheyenne	23	12.8	73.4		4886	Oct. 15, 1907
	Do	11	19.3	82.8		4891	Oct. 23, 1907
	Chugwater	25	15.9	80.9	Red—small	5213	Dec. 3, 1907
	Do	10	6.4	55.1	do	5225	Do.
	Cheyenne	18	16.0	81.6	Good	5705	Oct. 6, 1908
	Do	16	24.3	92.0	Fair	5773	Oct. 15, 1908
	Sheridan						
	Kendrick	14	9.5	68.8	Poor	5804	Oct. 22, 1908
	Weston						
	Cambria	8	17.8	86.2	Fair	7137	Oct. 2, 1909
	Horton	14	11.2	79.0	do	7939	Oct. 18, 1910
	Uinta						
	Lyman	26	15.7	83.1	Good	5780	Oct. 17, 1908

YEARLY AVERAGE ANALYTICAL DATA BY STATES, FROM 1884 TO 1900, AND FROM 1905 TO 1910.

The following table contains the yearly average analytical figures of the bureau for beets grown in the various States since 1884, with the exception of the years 1901–1904. The figures are of value in showing the variability of the sugar content and purity by years and in providing a basis for a comparison of the various States as sugar-beet producers. Unlike the preceding table, the sugar content has been figured by a factor to the sugar in the beet. The results for the years up to 1898 are taken from Bulletin No. 52 of the Bureau of Chemistry, while the results for 1898, 1899, and 1900 are taken from the Report of Progress of Beet Sugar Industry in the United States for these years. The results for 1905 to 1910 are based on the figures given in this report.

Yearly average of analyses of beets, by States, made in Bureau of Chemistry, 1884 to 1900 and 1905 to 1910.

State and year.	Number of samples.	Average weight.	Sugar in beet.[1]	Purity.
		Ounces.	*P. ct.*	
Alabama:				
1893	......	......	5.9	66.7
1898	4	18	8.5	73.2
1909	1	13	12.4	77.6
Arizona:				
1891	2	51	7.7	56.9
1897	7	23	9.3	70.4
1908	1	16	13.9	77.2
Arkansas:				
1891	2	40	6.4	58.8
1892	3	12	9.4	64.7
1897	2	18	11.3	71.5
1898	6	23	7.1	67.5
1899	5	15	6.0	55.5
1900	2	9	6.7	61.6
1906	1	8	10.8	80.3
1907	1	5	14.4	78.8
1909	1	16	12.3	......
1910	4	16	10.9	75.4
California:				
1884	71	19	13.7	85.3
1890	4	13	14.7	84.6
1891	8	48	11.1	75.8
1892	4	14	14.7	77.6
1897	1	26	16.8	......
1898	4	25	14.6	80.2
1899	1	11	13.9	82.0
1900	4	13	12.9	78.9
1908	6	13	13.3	82.2
1909	7	23	13.5	79.9
1910	2	32	16.8	82.5
Colorado:				
1890	29	20	12.5	76.1
1891	51	26	13.1	76.1
1892	170	18	14.8	81.7
1893	18	17	13.2	74.9
1897	174	20	13.6	76.7
1898	50	22	13.7	80.1
1899	64	24	14.4	80.2
1900	57	25	14.1	78.7
1905	22	29	16.1	82.6
1907	2	19	19.7	86.7
1908	9	17	13.6	79.5
1909	24	35	16.7	82.8
1910	4	20	11.9	70.9
Connecticut:				
1890	2	14	9.7	76.1
1891	5	27	10.8	77.3
1898	4	21	10.3	76.2
1899	2	17	10.9	75.5
1900	1	20	10.0	73.4
Delaware.				
1898	1	14	11.3	78.8
1899	2	24	12.5	81.4
1900	1	15	10.0	75.6
Georgia:				
1891	2	12	11.1	64.9
1898	4	47	5.8	64.0
1899	10	14	11.0	75.0
1900	1	10	14.2	86.6
1908	2	33	9.6	73.7
1909	1	14	8.5	71.6
1910	2	11	9.6	73.2
Idaho:				
1890	1	4	8.0	68.3
1891	1	15	12.7	74.9
1892	2	34	14.7	79.1
1893	2	78	10.2	76.2
1897	7	21	15.5	79.4
1898	5	28	12.0	78.3
Idaho—Continued.				
1899	1	36	10.8	75.0
1900	19	26	13.5	81.4
1906	1	24	19.1	89.5
1910	2	27	16.3	83.5
Illinois:				
1890	8	31	10.3	72.1
1891	36	32	11.7	76.4
1892	59	15	10.9	75.2
1897	32	17	13.1	75.5
1898	38	20	10.2	75.2
1899	25	25	10.6	72.6
1900	16	27	8.3	65.2
1905	7	20	11.6	76.1
1906	27	19	16.0	81.6
1908	1	12	18.8	88.0
1909	7	29	12.0	76.2
1910	3	41	9.6	71.5
Indiana:				
1890	56	23	10.7	72.7
1891	77	27	11.6	76.9
1892	57	14	11.2	72.5
1893	4	10	10.7	73.1
1897	103	14	13.1	78.9
1898	88	21	10.1	75.5
1899	29	19	11.4	73.4
1900	15	21	9.4	71.1
1909	3	26	11.6	75.6
1910	2	42	11.1	74.8
Indian Territory:				
1891	1	27	11.6	75.9
1898	1	27	9.6	77.1
1900	3	29	7.9	66.2
Iowa:				
1890	30	22	11.8	74.5
1891	321	30	11.8	75.7
1892	30	24	10.9	76.2
1893	7	17	12.8	75.8
1897	130	18	13.3	73.7
1898	147	25	11.4	76.1
1899	67	24	10.9	72.1
1900	39	33	9.5	70.7
1905	16	24	11.3	76.1
1908	2	28	14.8	87.7
1909	10	30	11.3	75.2
Kansas:				
1890	22	32	8.3	69.3
1891	36	33	10.7	68.2
1892	22	25	11.1	74.2
1893	1	......	14.3	72.8
1897	41	27	11.4	73.8
1898	16	22	10.3	71.3
1899	35	22	9.6	66.0
1900	20	25	10.1	72.1
1907	11	19	10.8	72.3
1908	6	28	11.4	75.2
1909	1	24	15.3	82.1
1910	3	7	15.7	78.5
Kentucky:				
1891	3	34	9.1	63.7
1892	4	13	8.9	77.2
1897	6	16	11.9	71.5
1898	4	14	5.9	61.1
1899	1	5	7.4	......
1900	12	13	8.5	64.8
Louisiana:				
1893	3	12	10.3	72.2
1910	1	5	9.0	70.8
Maine:				
1900	2	19	8.1	67.7

[1] Calculated from sugar in juice by factor.

Yearly average of analyses of beets, by States, made in Bureau of Chemistry., 1884 to 1900 and 1905 to 1910—Continued.

State and year.	Number of samples.	Average weight.	Sugar in beet.	Purity.
		Ounces.	*P. ct.*	
Maryland:				
1890	83	15	12.2	79.3
1891	2	16	7.4	68.5
1897	29	19	11.4	79.1
1898	31	22	10.4	76.0
1899	6	18	10.2	74.6
1900	1	10	9.3	74.2
1905	1	21	16.6	79.8
1910	9	15	14.5	84.0
Massachusetts:				
1890	6	16	12.0	82.8
1898	4	27	12.0	78.6
1899	9	21	14.6	83.3
1900	2	8	14.0	......
Michigan:				
1890	30	31	12.0	78.4
1891	50	32	12.6	78.0
1892	71	19	14.1	83.4
1893	88	15	13.3	82.1
1897	450	22	14.7	81.1
1898	34	28	13.2	81.9
1899	236	22	13.1	79.7
1900	478	14	11.3	76.7
1905	21	15	16.4	82.8
1906	28	22	15.9	83.5
1907	2	18	15.6	86.1
1909	2	17	16.0	86.0
Minnesota:				
1890	107	30	11.8	75.2
1891	41	29	12.4	75.7
1892	22	29	12.2	78.1
1893	7	60	10.8	70.8
1897	49	24	11.0	79.2
1898	21	22	12.7	78.7
1899	9	23	12.3	77.5
1900	10	31	10.9	75.9
1910	1	9	9.3	66.6
Mississippi:				
1898	2	20	4.2	65.7
1908	1	....	12.4	78.3
1909	5	13	9.9	69.9
1910	1	20	9.2	72.7
Missouri:				
1890	2	21	8.4	66.7
1891	67	20	10.4	62.4
1892	13	33	8.1	63.4
1897	324	20	11.7	73.5
1898	43	17	8.5	68.6
1899	19	17	7.1	64.3
1900	9	15	8.5	67.2
1908	3	13	12.2	79.7
1909	5	16	10.5	72.4
1910	4	10	8.7	69.9
Montana:				
1891	35	25	13.2	76.8
1892	6	22	10.9	72.8
1893	2	15	14.3	75.0
1897	4	20	14.4	77.8
1898	7	21	11.2	72.6
1899	2	40	10.7	70.6
1900	4	38	10.4	69.3
1909	1	24	16.0	76.8
1910	2	14	15.9	86.1
Nebraska:				
1890	269	20	11.8	71.9
1891	62	35	11.7	75.3
1892	27	21	14.2	79.3
1893	8	17	10.1	69.7
1897	13	29	12.9	76.9
1898	10	25	12.8	76.8
1899	6	19	11.3	74.4
1900	11	28	9.9	72.1
1908	5	19	9.1	68.3
1909	4	23	15.0	80.8
Nevada:				
1891	18	11	17.2	88.0
1892	81	13	15.9	83.4
1897	21	18	18.3	81.4
1898	42	12	18.5	85.9
1899	24	18	16.9	82.2
1900	3	10	9.6	79.6
Nevada—Contd.				
1908	5	22	16.0	85.8
1909	13	23	17.5	87.4
New Hampshire:				
1891	1	19	11.6	80.0
1898	2	34	13.5	83.5
1899	4	17	15.5	86.0
1900	9	29	11.1	74.9
New Jersey:				
1891	1	17	7.3	70.8
1897	31	16	14.2	81.4
1898	33	20	11.1	77.5
1899	17	27	11.3	77.3
1900	2	21	11.4	76.7
1909	1	16	7.9	63.5
New Mexico:				
1891	17	28	13.8	74.8
1892	29	19	15.3	83.2
1897	3	13	17.2	82.0
1898	7	20	12.8	78.0
1899	2	22	14.9	82.9
1900	2	23	14.5	77.8
1906	2	20	15.3	78.1
1907	20	21	17.9	83.2
1908	51	19	15.4	84.7
1909	14	19	14.6	80.1
1910	1	17	16.0	77.0
New York:				
1890	10	15	12.1	78.0
1891	4	32	11.6	76.8
1892	8	22	15.4	85.9
1897	225	21	15.0	82.4
1898	328	21	12.6	80.5
1899	142	19	13.0	78.8
1900	51	22	13.3	79.8
1910	2	10	13.8	83.5
North Carolina:				
1892	4	4	9.0	73.4
1897	7	23	9.1	75.3
1898	14	19	6.5	61.8
1899	2	17	7.6	69.0
1900	4	23	10.3	76.4
1907	7	17	12.2	80.8
1908	2	26	9.3	72.6
1909	4	14	14.0	82.6
1910	1	24	13.5	82.7
North Dakota:				
1890	24	25	13.4	71.2
1891	11	23	11.8	73.2
1892	11	24	12.9	76.5
1893	2	27	14.0	80.7
1897	4	28	10.5	81.2
1899	3	22	13.9	78.3
1900	5	22	10.2	71.1
1905	6	35	12.7	73.9
1908	7	15	14.4	83.6
1910	4	24	13.9	76.9
Ohio:				
1890	15	26	9.8	76.0
1891	66	31	11.3	73.5
1892	102	17	14.2	80.2
1897	68	22	13.8	79.1
1898	409	24	11.0	77.1
1899	128	24	11.9	76.1
1900	64	26	10.3	73.1
1906	5	27	17.0	81.7
1907	15	14	18.1	87.8
1908	1	16	9.5	73.0
1909	2	18	15.6	85.2
1910	2	19	11.9	75.0
Oklahoma:				
1891	1	48	6.4	53.3
1897	1	10	11.8	72.5
1898	6	24	10.2	73.3
1899	2	31	10.3	69.3
1900	4	22	10.6	69.8
1907	3	23	14.7	78.1
1908	7	25	13.8	81.2
1909	16	15	12.0	74.5
1910	4	19	13.4	81.9

Yearly average of analyses of beets, by States, made in Bureau of Chemistry, 1884 to 1900 and 1905 to 1910—Continued.

State and year.	Number of samples.	Average weight.	Sugar in beet.	Purity.
Oregon:		*Ounces.*	*P. ct.*	
1890	2	20	15.1	73.4
1891	35	34	12.7	81.1
1892	12	19	14.2	80.2
1898	6	20	14.1	83.4
1899	1	17	15.8	84.3
1900	11	19	12.4	78.9
1905	2	16	14.3	81.0
1906	1	32	12.2	68.0
1907	3	26	17.1	83.1
1908	3	17	17.0	86.2
1910	15	21	15.6	81.3
Pennsylvania:				
1890	10	27	8.0	73.8
1891	7	22	13.3	78.7
1892	8	13	10.8	75.8
1893	1	....	11.0	78.9
1897	59	18	13.8	79.5
1898	81	21	11.6	78.1
1899	28	31	11.2	75.4
1900	11	21	10.6	74.8
1907	1	13	15.3	79.7
1908	3	18	14.2	82.4
1909	1	12	14.2	81.4
Rhode Island:				
1897	2	21	11.9	74.2
South Carolina:				
1897	13	17	9.9	79.9
1898	4	14	10.2	81.2
1899	4	14	13.0	79.3
1900	3	29	8.4	69.5
South Dakota:				
1890	21	20	13.1	78.6
1891	202	22	12.5	75.3
1892	67	20	13.1	75.5
1897	5	17	15.1	83.2
1898	24	16	13.9	78.6
1899	11	25	10.6	72.8
1900	9	29	10.5	71.7
1907	1	16	17.1	75.3
1909	3	13	16.6	82.8
Tennessee:				
1891	5	20	8.8	65.8
1892	1	10	9.4	72.4
1897	17	11	10.8	71.9
1898	10	17	8.0	69.3
1899	2	54	8.3	67.6
1900	1	15	5.4	54.8
1909	14	15	9.7	71.1
1910	2	25	10.3	72.8
Texas:				
1890	2	38	10.0	69.3
1891	10	23	10.3	69.1
1897	11	22	12.6	76.5
1898	49	25	9.5	69.8
1899	3	12	7.5	53.7
1900	15	29	6.9	56.8
1905	24	27	12.5	75.6
1906	17	27	13.2	75.8
1907	8	19	14.3	73.3
1908	24	20	12.7	77.6
1909	26	15	15.1	77.6
1910	10	20	13.1	76.9
Utah:		*Ounces.*	*P. ct.*	
1897	35	20	14.3	81.1
1898	14	16	13.6	85.3
1899	10	20	15.0	83.6
1900	5	14	13.8	81.3
1908	1	35	15.2	90.9
1909	4	8	13.2	79.4
1910	11	23	14.9	82.7
Vermont:				
1897	8	22	14.2	84.1
1898	68	22	13.2	82.8
1899	16	23	12.8	79.0
1900	3	15	12.1	79.7
Virginia:				
1890	20	15	10.8	74.0
1891	72	21	11.1	76.0
1892	13	12	12.0	79.6
1893	14	16	13.3	83.9
1897	34	21	11.6	76.2
1898	43	20	8.9	72.4
1899	6	17	9.5	74.2
1900	49	18	10.0	74.1
1907	2	16	14.7	81.2
1908	55	21	14.4	83.7
1909	51	19	12.9	79.1
1910	6	20	11.3	78.2
Washington:				
1890	1	16	15.2	84.2
1891	11	18	14.5	83.9
1892	31	18	14.5	76.8
1893	183	28	12.3	74.0
1897	34	27	13.7	80.7
1898	5	27	13.9	81.3
1899	8	23	13.0	77.8
1900	2	24	14.9	85.6
1910	1	9	18.3	89.0
West Virginia:				
1892	12	14	11.3	68.5
1897	14	19	15.4	80.4
1898	4	28	9.1	72.9
1899	3	20	9.1	67.2
1900	4	7	10.7	62.7
1909	1	16	11.7	70.1
1910	16	22	13.2	77.1
Wisconsin:				
1890	10	21	12.8	81.3
1891	432	26	11.1	75.8
1892	21	22	12.7	77.8
1897	42	15	15.8	83.3
1898	16	24	13.0	79.3
1899	25	21	14.8	84.4
1900	18	30	10.0	73.1
Wyoming:				
1890	5	26	15.1	78.8
1891	18	12	13.5	78.1
1892	6	8	15.2	85.2
1893	48	19	15.9	80.5
1897	34	19	17.2	82.3
1898	10	19	13.9	78.1
1899	1	29	15.9	81.9
1900	2	20	13.5	78.5
1907	4	17	13.0	73.0
1908	4	19	15.5	81.4
1909	2	20	14.0	80.1
1910	2	18	12.4	79.2

BIBLIOGRAPHY OF CHEMICAL LITERATURE ON METHODS OF ANALYZING BEETS (1839 TO 1906, INC.).

This bibliography does not pretend to be complete, but it contains titles of papers that have been noted by the author in a search through the complete files of the Pharmaceutische Centralblatt, Chemisches Centralblatt, Zeitschrift des Vereins der Rubenzuckerindustrie and Zeitschrift des Vereins der deutschen Zuckerindustrie, Scheibler's Neue Zeitschrift der Rubenzuckerindustrie, Zeitschrift für angewandte Chemie, La Sucrerie Indigene, and of certain volumes of the Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie, Die deutschen Zuckerindustrie, Osterreichische-Ungarische Zeitschrift für Zuckerindustrie und Landwirtschaft, and Zeitschrift für Zuckerindustrie in Böhmen.

After having completed this review, a volume by E. O. Von Lippmann on "Die Entwicklung der deutschen Zuckerindustrie von 1850 bis 1900" was found in which the author devotes some 10 pages to abstracts, with references, of articles on analysis and valuation of sugar beets. The papers referred to there, with few exceptions, are noted in this bibliography. The list is made by years, with the authors' names in alphabetical order. It has been brought up to 1907, as from that time on such literature will be found in the volumes of Chemical Abstracts, American Chemical Society.

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H. W. WILEY, CHIEF OF BUREAU.

COAL-TAR COLORS USED IN FOOD PRODUCTS.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., January 31, 1911.

Sir: I have the honor to transmit for your approval a report by Bernhard C. Hesse, a color expert, containing both original chemical work done in the bureau since the passage of the food law on anilin dyes used for foods, and a valuable and extensive compilation of the literature of the subject, especially with reference to the harmfulness of coal-tar colors and their physiological effects. These data formed the basis of the opinions stated in Food Inspection Decisions 76, 77, and 106, and are presented in detail as of scientific and practical interest to all those concerned in the use of coal-tar colors in foods, whether as manufacturers, food officials, or consumers. I recommend the publication of this report as Bulletin No. 147 of the Bureau of Chemistry.

Respectfully,
H. W. Wiley, *Chief.*

Hon. James Wilson,
Secretary of Agriculture.

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COAL-TAR COLORS USED IN FOOD PRODUCTS.

INTRODUCTION.

PURPOSE OF THE INVESTIGATION.

For the purposes of the investigation reported in the following pages, the legitimacy of the coloring of food and food products under certain conditions is regarded as established; the ethical and dietetic aspects of the question of food coloring are not here considered.

The means at hand for coloring food products may be conveniently classified as vegetable, animal, mineral or inorganic, and synthetic or so-called coal-tar colors or dyes. Representatives of each of these have at one time or another all been used in the coloring of food, and the laws of various European and American States have, from time to time prohibited the use of certain specified members or all of each or some of the foregoing classes. It is therefore obvious that even for the legitimate purposes for which food can be colored, improper means are at command, and some of these, if not all, have been prohibited by law at some time or another.

It is the function of the present work to determine what members of the synthetic or coal-car colors should be considered legitimate for coloring foods. It is confidently believed that the material collected in the following pages points clearly and solely to the following conclusions:

1. Coal-tar dyes should not be used indiscriminately in foods.
2. Only specified coal-tar dyes should be used in foods.
3. Only tested and certified dyes should be used in foods.

The work here reported has furnished the basis for Food Inspection Decisions Nos. 76, 77, and 106, issued July 13, 1907, September 25, 1907, and March 25, 1909, respectively, and the investigation itself was practically terminated January 1, 1910.

The effect of these decisions has been to restrict the coal-tar colors permitted for use in foods to seven specified and enumerated colors, until such time as it shall be shown with reasonable conclusiveness that other colors should be added to such list; and further, all coal-tar colors permitted for use in food are to be of a degree of purity and cleanliness acceptable to the Department of Agriculture, and are to be so certified.

In order to avoid any uncertainty as to the chemical composition of the enumerated colors, direct reference is made in Food Inspection Decision No. 76 to a standard work in which such chemical composition is clearly and unequivocally set forth. The relevant parts of Food Inspection Decision No. 76 are as follows:

> The use of any dye, harmless or otherwise, to color or stain a food in a manner whereby damage or inferiority is concealed is specifically prohibited by law. The use in food for any purpose of any mineral dye or any coal-tar dye, except those coal-tar dyes hereinafter listed, will be grounds for prosecution. Pending further investigations now under way and the announcement thereof, the coal-tar dyes hereinafter named, made specifically for use in foods, and which bear a guaranty from the manufacturer that they are free from subsidiary products and represent the actual substance the name of which they bear, may be used in foods. In every case a certificate that the dye in question has been tested by competent experts and found to be free from harmful constituents must be filed with the Secretary of Agriculture and approved by him.
>
> The following coal-tar dyes which may be used in this manner are given numbers, the numbers preceding the names referring to the number of the dye in question as listed in A. G. Green's edition of the Schultz-Julius Systematic Survey of the Organic Coloring Matters, published in 1904.
>
> The list is as follows:
>
> *Red shades:* 107. Amaranth. 56. Ponceau 3 R. 517. Erythrosin.
> *Orange shade:* 85. Orange I.
> *Yellow shade:* 4. Naphthol Yellow S.
> *Green shade:* 435. Light Green S F Yellowish.
> *Blue shade:* 692. Indigo disulfoacid.
>
> Each of these colors shall be free from any coloring matter other than the one specified and shall not contain any contamination due to imperfect or incomplete manufacture.

The reasons, broadly considered, which led up to these food inspection decisions are given in concise fashion in this introduction.

Looking over the restrictions placed upon coal-tar colors by the lawmakers of the various countries it will be found that certain colors are in some instances specifically prohibited and in other instances that certain specific colors, or classes of colors, and only such, are permitted for the legitimate purposes of food coloring.

Private organizations, such as the Swiss Society of Analytical Chemists and the National Confectioners' Association in the United States, have also made recommendations permitting specific colors only, and in addition specifically prohibiting others. Individual authors have likewise made similar recommendations. The control of the quality of the food colors practiced on the part of those Governments which restrict the use of coal-tar colors to certain individuals, so far as any publications show, has not been very extensive.

The action taken against the use of coal-tar colors for food-coloring purposes has ranged all the way from absolute prohibition of their use for any purpose whatsoever to the practically unlimited use in legitimate food coloring operations of all but two of such colors.

Intermediate between these two extremes we find the prohibition of a greater number than two, or of all the members of this class except certain specified colors, and even here with the restriction that they shall be used only for certain legitimate food-coloring purposes.

It would be desirable to have a number of coal-tar colors of established harmlessness specifically permitted, particularly if the number be sufficient to meet all the legitimate demands arising in the food-coloring art. To prohibit only specified coal-tar colors and, by implication, to permit all the rest of this class, would allow the unrestricted use of newly discovered colors, and all other coal-tar colors not examined as to their effect on health. A limited list of permitted coal-tar colors which would make the use of all coal-tar colors outside of the permitted list illegal would properly protect the health and could work no substantial hardship upon those engaged in food coloring. Any such hardship would be avoided by providing that if it is shown that none of the colors of the permitted list meets certain legitimate requirements and that coal-tar colors outside the permitted list are capable of satisfying this need and are in and of themselves harmless the permitted list can be expanded by the proper authorities to meet additional needs or growing requirements without exposing the public health to any risk.

NUMBER OF COLORS PERMITTED.

It will be shown in the following pages that in the summer of 1907 there were on the market of the United States 80 different chemical individuals, or so-called coal-tar colors, offered for the coloring of food. It has been known since 1888 that it is unsafe to attempt to predict the harmfulness or the harmlessness of coal-tar colors by inference or analogy; therefore an ideally perfect permitted list should contain only such colors as have each been examined physiologically, separately, and specifically, and their harmlessness determined by actual test. Out of the 80 colors referred to 30 had not been examined at all, so far as the literature shows, and therefore their harmlessness is certainly open to question; 26 had been examined physiologically, and the published accounts with respect to their harmlessness or their harmfulness are in each case contradictory; on 8 none but adverse reports were to be found in literature, leaving only 16 out of 80 colors on the market which had been established with more or less certainty as harmless—that is, the users of these 8 colors were deliberately taking chances with the public health, since the harmful nature of those 8 had for a long time past been known to those conversant with such subjects; the use of the 26 doubtful colors is more defensible than the use of the 8 known to be harmful. Out of the 30 of whose action nothing was known it can not be said how many are or are not harmful, nor can the risk forced upon the public health be satisfactorily measured.

This brief summary must suffice for the present as a justification for the restrictions of the permitted colors to 7 in number. The full reasons for each and every step will appear in their proper places in the pages following.

QUALITY AND EFFICIENCY OF COLORS PERMITTED.

An examination of 30 specimens representative of the 7 selected permitted colors on the United States market in the summer of 1907 disclosed such a condition of uncleanliness of product, or careless or improper manufacture, and the use of such utterly inferior qualities of products for food coloring purposes, that control over the quality of the seven permitted colors seemed necessary. The results of the work in the making and maintaining of standards of quality for each of these seven colors also justify this control. That there was in 1907, and for a year or more later, a considerable divergence of opinion among chemists as to what should be the proper quality requirements for these colors is shown by the fact that out of 72 foundation certificates offered in accordance with Food Inspection Decisions Nos. 76 and 77, 57 were rejected on their face because they did not comply with the standards of quality then in mind, or then shown to be commercially attainable. Much objection has been made by many of those whose certificates were rejected on the ground that the standards then in mind were unreasonable, unjustifiable, and nonattainable. The actual results, however, are that with very few exceptions the standards in mind early in the work have all been exceeded in practice; the 41,000 pounds (20.5 tons) of certified colors now in existence made in 97 batches, or an average of more than 420 pounds per batch, are, with the exception of perhaps one or two first batches, far cleaner than was expected when the 57 certificates above referred to were rejected.

There has been no complaint against the permitted colors for want of efficiency or for the possession of unsuitable attributes, which has been pressed or sustained with any such earnestness as would reasonably be expected if the defects complained of were as great as they were represented. Complaints have been made against the yellow, when used in acidulated fruit sirups, on account of its possessing a bitter taste; the proof of this, so far as any has been offered, was for a long time not of a convincing nature, and it was two years after the first objection was raised before any concerted or positive action was taken by those interested. The yellow has also been criticized because it is not sufficiently fast to light; although it was satisfactorily shown that another yellow was faster to light than the permitted yellow, no one has maintained that the yellow desired was actually preferable to the permitted yellow wholly and solely because of its superior fastness to light.

The blue has been criticized because it is not of the proper shade to permit of its use in the bluing of sugar, but the substitute offered therefor has not been supported by its sponsors in a way to indicate that a defect of serious magnitude exists. On the grounds of sufficiency and of efficiency the list of permitted colors selected appears to have been justified by the absence of any real or substantial complaint against them, on either or both of these grounds, during a period of more than three years.

None of the seven permitted colors is patented; their manufacture and their purification are open to all, and none of the 80 colors on the market in the summer of 1907, with perhaps one exception, had been discovered since 1891; in other words, the advances in the coal-tar industry from 1891 to 1907 had added nothing to the colors serviceable to the art of food coloring.

The list of colors permitted in Food Inspection Decision No. 76 embraces, therefore, a sufficient number of colors for all legitimate food-coloring purposes, the coloring of fats, oils, butter, etc., excepted, for which no suitable color had been examined and reported in the literature as being harmless and fit for use in foods; they can be made by any one; no one can have a monopoly in any one of them by virtue of patents; any competent maker can make all or any of them and purify them to the required degree of cleanliness. The standards growing out of the control exercised by the Department of Agriculture are such as to insure that the colors used for food-coloring purposes possess a proper degree of cleanliness and such a degree of cleanliness is commercially feasible and is a commercial reality.

The policy adopted in this respect is therefore justified not only from the viewpoint of the history of the attempts on the part of various governments to control the quality of food colors, but also by the results actually obtained by its adoption. This policy of restricting food colors to certain chemical individuals and demanding that those possess certain qualities is in complete harmony with the following suggestion made to the commission on rules and regulations under the food and drugs act, at its hearing in New York, in September, 1906.

> Any kind of a harmless color should be permitted, provided it is not a color generally known to be poisonous, or generally found to be poisonous, or one that may be almost impossible to be produced without containing some poison within itself when finished and ready for use. Coal-tar colors, as a class, should not be prohibited, but all those coal-tar colors generally found to be poisonous, or which are hard to produce without containing some poisonous properties when ready for use, should be forbidden the privilege of being used, or offered for sale for use in food.
>
> Under the provisions of section 2, we have this to recommend to the commission, that every person selling or using a coal-tar color in food or drink, should be required

to secure, either on his own account, or from the person from whom he buys such color, a certificate to the effect that the identical color used has been tested for poisonous ingredients, and is, to the knowledge of the chemist making the test, absolutely harmless. The chemist should be required to be a competent physiological chemist, and must certify as above under oath. This would mean not that each package of color would have to be tested, but that every batch would have to be tested, and the certificate would then be held to relate to every batch. Such tests should be made in the United States and the chemist certifying should reside in and be a citizen of the United States.

It should not be deemed sufficient to have any particular brand of coal-tar color tested once, and a blanket certificate given, covering the whole brand as long as it may be sold, but every ounce of coal-tar color put out by a color manufacturer should be shown by actual test to be harmless.

While the exact mode of reaching the end in view is somewhat different from the one suggested above, yet the fundamental object, that each batch of color used in foods shall be specifically tested, and that such colors shall be harmless, is attained with reasonable certainty; and although there are colors other than these seven which are undoubtedly equally as little objectionable, and while it is true that the present policy contemplates the permitted use of but seven specific colors, yet that policy, as before outlined, is sufficiently broad and elastic to enable the addition of a color to the permitted list, when it is shown that such color really fills a need, not properly satisfied by one of the colors already permitted or some combination of these, and is in and of itself harmless. There can be no objection to the expansion of the list to such an extent as to include every harmless coal-tar color in existence; but the burden of proving such real need and harmlessness is very properly placed upon those who are seeking such expansion.

As far back as 1892 the following statement was made on page IV of the Leffmann translation of Weyl's book on coal-tar colors, in connection with the various European legislative enactments: "It is certain that none of these plans is even approximately satisfactory and the problem will be even more difficult of solution in the United States; indeed, it seems to me to be unsolvable." In view of this opinion the results of the food inspection decisions as herein shown may properly be regarded as, at least, a step in the right direction toward the solution of this problem.

This opinion is further supported by C. A. Neufeld who, in reviewing Food Inspection Decision No. 76, says: "The idea of permitting only specific selected coloring matters for use in the production of articles of food, and of excluding all other colors from such uses, must, in the interest of control of articles of food, be regarded as an extraordinarily happy one; a similar regulation is to be urgently recommended for our own country." (*Zts. Nahr. Genussm., 1908, v. 15, p. 434.*)

IDENTITY OF COAL-TAR COLORS USED IN FOOD PRODUCTS IN THE UNITED STATES IN 1907.

COLLECTION OF SAMPLES.

The question "Which coal-tar colors shall be permitted for use in coloring such food products as are to be consumed within the United States?" can be answered, "All colors now in use, or to be used for that purpose, provided they are harmless and necessary as defined on page 14."

This involves the further questions:

1. Which coal-tar colors of the 695 different chemical individuals now on the world's markets are actually used in the United States for that purpose?
2. If restricted to such coal-tar colors as are now in use in the United States for this purpose, would this be likely to hamper or interfere with the invention of other coal-tar colors suitable for the coloring of food?

It would be physically impossible to go to every user of coal-tar colors in food products in the United States and obtain specimens of the coal-tar colors so employed; this would be impracticable not only because of the large number of such users, and their wide geographical distribution, but also because they often do not know what they are using, and further because of a reluctance, undoubtedly to be encountered among many, to disclose the nature of the products employed. This is rendered more than likely by the attitude of some of the makers of coal-tar colors, or their accredited agents, as will be shown later.

However, the sources of coal-tar colors are limited in number. By reference to pages IX and X of "A systematic survey of the organic coloring matters," by Arthur G. Green, published in London and New York by Macmillan & Co. (Ltd.), in 1904 (hereinafter referred to as "Green Tables"), it will be seen that there are approximately 37 different concerns the world over engaged in the manufacture of coal-tar colors. Therefore a canvass of these sources for such coal-tar colors as in their judgment, or in their business practice, they regard as proper for use in food products, is the best way of arriving at a fair demarcation of the field of coal-tar colors here in question.

Communication was therefore had with 13 actual manufacturers of coal-tar colors, in an endeavor to obtain from them such coal-tar colors as in their judgment or business practice are suitable for use, or are used in food products.

A request was also made for information as to the chemical composition of the coal-tar color specimens submitted; in order to avoid confusion, it was further asked that reference be made to the Green Tables, in which each chemical individual or coal-tar color has its own number, and if any of the contributed specimens was not so listed that the chemical composition be stated in a manner analogous to that used in the Green Tables. This procedure was necessary in order to reduce the terminology to a common and nonequivocal basis. Out of the 13 makers, or their accredited sole importers or selling agents in the United States, who were consulted, 9 have supplied the specimens requested; the remaining 4 promised to contribute, but have not done so. In the following table is shown the amount and character of the information obtained:

Tabulation of distribution of replies and character of information received.

Country.	Geographical distribution.				Number of samples contributed.	Number of samples referred to Green Tables.	Number of samples not referred to Green Tables.	No composition or ambiguous terminology.
	Coal-tar color makers.	Makers asked to send samples.	Makers not sending samples.	Makers sending samples.				
Germany	16	6		6	181	106	1	74
England	8	1		1	12	12		
France	5	1	1					
Switzerland	5	3	3					
United States	1	2		2	61	35	5	21
Holland	1							
Belgium	1							
Total	37	13	4	9	254	153	6	95

In order to make provision for the 24 makers listed in the Green Tables and not included in the 13 makers addressed requests for samples were sent to two domestic houses which import coal-tar colors from scources other than the above, for use in food products; their products must fairly represent any of the colors not covered by the 13 makers addressed. Of these two importers, one responded with 13 samples, and of each he gave the number in the Green Tables corresponding to each specimen; the other importer has not redeemed his promise to contribute specimens.

A third importer volunteered the Green Table numbers of four out of five coal-tar colors used in his business, but could not even approximately say what the remaining color was chemically. He did not contribute any specimens, nor was that necessary at the time this information was volunteered.

A fourth importer contributed specimens of five coal-tar colors needed in his business, but was able to give Green Table numbers for only three of them; he could not give even approximately the chemical composition of the remaining two.

Out of the 17 responsible concerns consulted 5, or 29 per cent, have not found it to their interest to contribute either specimens or information.

CLASSIFICATION OF SAMPLES SUBMITTED.

GREEN TABLE NUMBERS.

Out of the 284 specimens contributed, or reported on, 172 (60.6 per cent) were identified as to their chemical composition, by reference to the Green Tables; 6 (2.1 per cent) were otherwise unequivocally identified chemically, and for 106 (37.3 per cent) the makers, or their responsible agents, declined to state the chemical composition, i. e., 62.8 per cent were unequivocally identified, and the remaining 37.3 per cent were not so identified.

The specimens submitted are therefore divisible into the following three classes:

Class I. Those for which numbers were given in the Green Tables, numbering 172.

Class II. Those whose composition was given in chemical language, numbering 6.

Class III. Those whose composition was not given in any language capable of correct and certain translation into chemical terms, numbering 106.

Consider Class I. The Green Tables, page VI, divide the coal-tar colors into 21 groups, comprising 695 different chemical individual coal-tar colors. The 172 members of Class I number in all 74 individuals, or 10.6 per cent of the Green Tables, and fall into 11 of the 21 groups of those Tables.

The following table classifies the samples according to the Green Table groups:

Green Table groups and number of collected samples falling within them.

Color groups of the Green Tables.	Number of members in group.	Collected samples falling into the several groups.		Color groups of the Green Tables.	Number of members in group.	Collected samples falling into the several groups.	
		Number.	Per cent.			Number.	Per cent.
Nitro	6	1	16	Anthracene	37	....	....
Monoazo	126	30	24	Indophenol	8	....	....
Disazo	204	11	5	Azin	38	2	5
Trisazo	46	....	....	Oxazin	32	....	....
Tetrakisazo	11	....	....	Thiazin	9	2	22
Nitroso	5	1	20	Thiazol	6	....	....
Stilbene	18	....	....	Quinolin	4	1	25
Oxyketone	8	....	....	Sulphid	21	....	....
Diphenylmethane	2	1	50	Indigo	7	1	15
Triphenylmethane	66	15	23				
Xanthene	35	9	26		695	74	10.6
Acridin	6	....	....				

SOURCE.

The distribution of the 74 different chemical individuals of Class I among the 12 different sources from which they were obtained is as follows:

Distribution of the 74 different samples of Class I among the 12 sources supplying same.

Number.	Per cent.	Number of sources from which each came.	Number.	Per cent.	Number of sources from which each came.
35	47.3	1	1	1.35	7
20	27	2	1	1.35	8
4	5.4	3	0	....	9
4	5.4	4	1	1.35	10
5	8.1	5	0	....	11
3	2.7	6	0	....	12

It follows from this table that there is very little unanimity among the different concerns furnishing coal-tar colors for use in food products as to which of their products are desirable, necessary, or suitable for such use.

Inspection of this table shows that only three colors out of 74, or 4 per cent, were wanted by more than half of all the sources; that only 6, or 8.1 per cent, were wanted by half of the sources; and that not one of the colors was wanted by all the sources. This last statement is true of manufacturers as well as importers, each group taken by itself.

PATENTS.

This lack of unanimity is not due to the patent situation, because not more than one of these 74 products is patented, and it is more than likely that the United States patent on this product has long since expired.

Moreover, only 6 of the 12 sources offered colors at one time patented by themselves or others. The total number of such ex-patented products is 45, and of these only 22 were offered by those who had patented them; the remaining 23 were offered by sources other than the ex-patentees, and were not offered by such ex-patentees.

Patented colors.

Total number of patented products offered.	Ex-patented products offered by patentee.	Total number of patented products offered.	Ex-patented products offered by patentee.
11	2	1	1
16	8	4	2
6	5		
7	4	45	22

It would, therefore, seem to be rather clear that others think more favorably of such ex-patented products as food colors than do the original patentees. In view of the fact that the latter would generally be in a better position, and would have greater opportunity than any one else to judge of the suitability of the patented products for use in food products, it may well be inferred that such products are not altogether free from disadvantages as food colors.

The second of the two questions propounded, namely, If restricted to such coal-tar colors as are now in use in the United States for this purpose, would this be likely to hamper or interfere with the invention of further coal-tar colors suitable for the coloring of food products? can be answered "No" because none of the colors submitted was discovered later than 1891; out of the 214 coal-tar colors since then discovered not one was among those submitted for use in foods, and out of the 481 discovered in 1891 and prior thereto, only 74 were so submitted, or 2 out of every 13 of such colors. In the following table these data are given year by year:

Coal-tar colors discovered from 1740 to 1891.

Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.	Year of discovery.	Number of submitted colors discovered.	Total number of coal-tar colors discovered.
1740	1	1	1871	1	6	1881	4	11	1887	1	29
1856	1	3	1874	1	4	1882	5	20	1888	1	36
1859	1	1	1875	6	12	1883	9	25	1890	1	46
1861	2	3	1876	5	9	1884	3	16	1891	3	33
1862	1	5	1877	2	10	1885	2	21			
1863	1	3	1878	12	26	1886	3	32	Total.	74	378
1867	1	3	1879	7	23						

SHADES OF COLOR.

The sufficiency of the 74 colors used for food-coloring purposes in the United States, for any and all tinctorial ranges, no matter how refined, appears from the following table:

Green Table numbers of the 74 submitted colors showing shades and number of sources supplying each.

Shades.	Number of sources out of a possible 12 offering each color.										Total number of dyes offered for each shade.
	1	2	3	4	5	6	7	8	9	10	
Red	104	65			106	103	107				12
	105	108									
	169	146									
	240	462									
	584										

Green Table numbers of the 74 submitted colors showing shades and number of sources supplying each—Continued.

Shades.	Number of sources out of a possible 12 offering each color.										Total number of dyes offered for each shade.
	1	2	3	4	5	6	7	8	9	10	
Blue red		502 518 520 523		448	504 517						7
Yellowish red	54 516	512									3
Scarlet	53 64	55									3
Bluish scarlet	56										1
Yellow	89 269 329			510	8	94				4	7
Reddish yellow	9	84									2
Orange yellow		14				13					2
Greenish yellow	667		425								2
Orange	18 97	17 85 95						86			6
Blue	439 476 480 655	650	692								6
Green blue	440										1
Violet	468	452			451						3
Blue violet	464										1
Grayish violet	287										1
Green	433 434	398		435							4
Blue green		427									1
Yellow green			428								1
Brown	101 137 139										3
Reddish brown		201		197							2
Blue black	188										1
Blue to bluish red to violet, according to brand	601										1
Oil-soluble colors	10 49 60	11									4
Total	35	20	4	4	5	3	1	1		1	74

The six colors comprising Class II (those not listed in the Green Tables but whose composition was avowed or disclosed) are one black, one yellow, and one orange among the water-soluble colors, and three yellows among the water-insoluble colors. Therefore two-thirds of the United States market as thus disclosed calls for a total of 80 different chemical individuals, of which 73 are water-soluble and 7 are water-insoluble and are used as oil or fat colors. The remaining third of the makers or dealers either do not possess the information or are unwilling to give it. No attempt has yet been made to enter systematically into this unknown region, but careful examination warrants the belief that it can add nothing of material value to the data already obtained which show a total of 23 shades for the 73 water-soluble coal-tar colors, summarized as follows:

Number of coal-tar colors.

5 red shades	26	3 green shades	6
4 yellow shades	14	2 brown shades	5
1 orange shade	7	1 blue-black shade	1
2 blue shades	7	1 black shade	1
3 violet shades	5	1 blue to violet shade	1

The water-insoluble colors numbering 7 are not included, but will be treated separately (p. 159).

This view of the state of the United States market at or about the middle of the year 1907 is without question a true reflection of that market as far as it goes and the actual extent of the coal-tar color market beyond those covered by this canvass of it is not likely to be very great. In support of this view is the interchangeable treatment of formerly patented products, the great lapse of time since a new food color was discovered, and the fact that out of the 74 colors submitted and contained in the Green Tables only 23 are now less than 25 years old, and none is less than 16 years old.

All the Green Table numbers and the number of sources out of a possible 12 offering them are given in the following table:

Number of sources, out of a possible 12, offering colors designated in 1907.

Green Table No.	Sources.	Green Table No.	Sources.	Green Table No.	Sources.	Green Table No.	Sources.
[1] *4*	10	86	8	240	1	476	1
8	5	89	1	269	1	480	1
9	1	94	6	287	1	502	2
10	1	95	2	329	1	504	5
11	2	97	1	398	2	510	4
13	6	101	1	425	3	512	3
14	2	103	6	427	2	516	1
17	2	104	1	428	3	*517*	5
18	1	105	1	433	1	518	2
40	1	106	5	434	1	520	2
53	1	*107*	7	*435*	4	523	2
54	1	108	2	439	1	584	1
55	2	137	1	440	1	601	1
56	1	139	1	448	4	650	2
60	1	146	2	451	5	655	1
64	1	169	1	452	2	667	1
65	2	188	1	462	2	*692*	3
84	2	197	4	464	1		
85	2	201	2	468	1		

[1] Italicized figures indicate colors permitted by F. I. D. 76.

Nine manufacturers sent 261 specimens, an average of 29 each, distributed as follows: 70; 38; 20; 20; 15; 43; 25; 18, and 12. Two importers sent 5 specimens each, and one 13, a total of 23 specimens, and an average of 8. These figures reflect a diversity of opinion as to what is needful for food coloring, since each one of these 12 makers or importers believed that for all practical food-coloring purposes his selection was complete and sufficient.

Classifying the 284 specimens as red, yellow, brown, orange, blue, green, violet, and black, the following table is obtained showing the different requirements of each of the 12 makers or importers to produce the necessary shades of the eight colors mentioned:

Total specimens submitted, grouped by makers and colors, showing number of shades required by each.

Maker's number.	Red.	Yellow.	Brown.	Orange.	Blue.	Green.	Violet.	Black.	Number of shades wanted by each.	Total specimens.
1	25	18	14	4	2	5	2	......	7	70
2	10	6	2	1	3	1	2	......	7	25
3	7	5	......	2	1	1	1	1	7	18
4	11	10	10	5	2	2	3	......	7	43
5	6	2	......	3	4	2	2	1	7	20
6	4	3	2	1	3	1	1	......	7	15
7	6	2	1	2	1	......	......	......	5	12
8	5	4	......	1	1	1	1	......	6	13
9	6	4	1	3	......	4	2	......	6	20
10	13	10	2	3	5	4	1	......	7	38
11	3	1	......	1	......	......	......	......	3	5
12	3	1	......	......	......	1	......	......	3	5
Total	99	66	32	26	22	22	15	2	......	284
Per cent	34.86	23.24	11.27	9.15	7.75	7.75	5.28	0.70	......	100
Maximum	25	18	14	5	5	5	3	1	......	......
Minimum	3	1	......	......	......	......	......	......	......	......
Average	8.25	5.50	2.75	2.17	1.83	1.83	1.25	0.17	......	......
Permitted	3	1	......	1	1	1	......	......	......	......

From this table it appears that not one of the 12 sources desired all of the 8 shades into which the 284 specimens are classifiable to make up a complete set of food colors; 7 out of the 12 sources wanted 7 of the 8 shades; 2 sources wanted 6 out of the 8 shades; 1 source wanted 5 of the 8 shades, and 2 sources were content with 3 out of the 8 shades.

It will be noticed that the permitted list given in Food Inspection Decision No. 76 provides for 7 dyes covering 5 out of the 8 shades of the above classification. The 3 missing shades are brown, violet, and black; the shades provided are red, yellow, orange, and blue.

It will also be noticed that on the whole 6 out of the 8 shades were not wanted by one or more of the 12 sources. The italicized shades are the ones not provided for by the permitted list of Food Inspection Decision No. 76.

Brown was not wanted by 5, nor orange by 1, blue by 3, green by 2, *violet* by 3, nor *black* by 10.

The combinations not wanted were as follows: Five sources omitted *black* only; 2, *brown* only; 1 blue and *black;* 1 *brown* and *black;* 1 green, *violet*, and *black;* and 1 *brown*, blue, green, *violet*, and *black*.

Not one of these 12 sources wanted only the three colors not found on the permitted list, and 2 sources did not want any of the three missing shades nor two of the permitted colors.

In view of this large difference of opinion among the 12 sources as to the shades needed to make a complete set of food colors, the 5 shades selected for the permitted list of Food Inspection Decision No. 76 seem reasonably close to any consensus of opinion derivable from the tabulation of the collected facts.

II. PURPOSES OF FOOD COLORING.

The use of any color which conceals inferiority, or which gives an article an appearance better than it properly possesses is, of course, illegitimate, and such cases are not here considered. Among such uses may be mentioned that of color in pastry to impart a yellow color thereto, implying the presence of eggs, when they are either wholly absent or are not present in sufficient quantities to produce a shade of color which would indicate a superior quality. Such coloring is frequently resorted to in macaroni, spaghetti, noodles, and the like, and it has also been stated in the literature that such coloring has the additional function of concealing dirt actually present in the flour.

The addition of red coloring matter to meat products to give them an appearance of freshness which they do not of themselves possess; the addition of red coloring matter to strawberry, raspberry, and similar jams, jellies, and preserves, to give them a color indicative of exceptional quality, even though they may contain none of the fruit whose presence is intimated by the label on the product; the injection of red coloring matter into ordinary oranges to give them the appearance of blood oranges; the sprinkling of lemons and oranges with green coloring matter to give them the appearance of a particular origin or of a particular state of ripeness when such origin or state of ripeness is without foundation in fact; the injection of red coloring matter into watermelons to give them the appearance of ripeness, which ripeness they do not possess, are practices met more or less frequently.

Among the purposes for which food colors are said to be used and the foods so colored, the following are mentioned in the literature:

In European countries.

1. Macaroni is colored with Dinitrocresol (2) (*Arch. Pharm., 3d ser., v. 22, p. 621*) and Martius Yellow (3) (*Weyl, Handbuch*).
2. Cordials and liqueurs with Dinitrocresol (2) (*Arch. Pharm., 3d ser., v. 22, p. 621*).

3. Oranges: Biebrich Scarlet (163) (*Weyl, Handbuch*).
4. Pastry: Dinitrocresol (2) (*Weyl, Handbuch*).
5. Butter: Dinitrocresol (2) (*Weyl, Handbuch*).
6. To whiten flour: Anilin blue (457) (*Zts. Nahr. Genussm., 1906, v. 12, p. 298*).
7. Noodles are colored to cover up cigar butts, burnt matches, mineral oil, etc. (*Zts. Nahr. Genussm., Vol. II, p. 1018*).

In the United States.

8. Jellies, fruit sirups, soda sirups, jams, ketchup, cheap cordials, lemon extract, milk, butter, cheese, ice cream, confectionery, pastries, flavoring extracts, mustard, cayenne pepper, sausage, noodles, wines, and liqueurs (*Winton, Connecticut Agricultural Experiment Station Report, 1901, pp. 179–182*).
9. Cattle feed is colored yellow (*Gudeman, J. Amer. Chem. Soc., 1908, v. 30, p. 1623*).
10. "Egg color" (399); "Macaroni color" (94); "Tomato catsup color" (105); "Raspberry color" (103); "Mustard color" and "Pie filling color" (4); "Orange color" (87), and "Strawberry red color" (55) are corresponding United States commercial food color names and their corresponding Green Table numbers (*Meyer, J. Amer. Chem. Soc., 1907, v. 29, p. 895*).

Dr. E. Ludwig, of Vienna, stated, upon the authority of Dr. Schacherl, at the International Congress of Medicine held in Budapest, in August, 1909, as follows:

> The rather widely distributed practice of coloring baker's goods yellow, such as cakes and the like, further the yellow coloring of pastry, macaroni, noodles, and so forth, has as its function the representation of a very large egg content in them; this coloring has been made very convenient because there are in commerce colors intended specifically for this purpose and designated "egg substitute" and which have nothing whatever in common with egg yolk.
>
> Marmalades such as apricot, raspberry, and currant marmalades are frequently found in a colored condition in commerce; in this case the purpose of the coloring is frequently to cover up adulteration; the adulteration may consist in an admixture of a cheap fruit pulp, particularly apple pulp, or in an addition of glucose sirup. Since these admixtures do not possess the color of the marmalades they are simply helped along by the aid of color.
>
> Old fruit sirups are toned up with color and then sold as fresh sirup.
>
> Red colored fermentation vinegar and red colored vinegar essence as well as vinegar made from such essence are in commerce; such coloring has for its purpose to represent the product as "genuine red wine vinegar," which in some countries is highly desired.
>
> So-called "beer color," said to be an extract of roasted malt (malt caramel), is in fact nothing but ordinary sugar caramel and is frequently from time to time publicly advertised; breweries themselves do not use this preparation, but it has been frequently shown that in small taverns by means of this color local beer was converted into Bavarian beer.
>
> The wholesale coloring of coffee beans serves the purpose of representing a better quality than it actually is.
>
> The coloring of cocoa and chocolate by the use of mineral additions and also of coal-tar colors was often proven; in this case the coloring serves exclusively to cover up poor quality. In the case of good products such coloring is not practiced.
>
> Colored sausages, and in fact such with a colored meat body as well as such with a colored casing, are frequently colored; coal-tar colors and cochineal serve this purpose, the latter, however, only for the meat. This coloring is to preserve in old goods the appearance of fresh goods.
>
> The green canned goods of commerce are almost all colored with copper compounds.

Tomato pulp frequently comes into commerce colored with a coal-tar color; the purpose of such coloring is to impart to the goods the appearance of having been prepared with extraordinary care. In all these cases it is not at all a question of a harmless change of the natural condition of the food product, but of improper manipulations which are adapted to deceive the purchaser as to the real value of the goods; even, indeed, to mask the danger to health.

III. FOOD-COLOR REQUIREMENTS.

ADAPTABILITY FOR SPECIAL PURPOSES.

Not all coal-tar colors are adapted for use in food products. Colors are the more desirable for this purpose the higher their tinctorial power, and the greater the resistance they offer to the action of the materials with which they are to be used, and under the conditions existing. Obviously only such colors as of themselves have their tinctorial properties fully developed can be used, and all such colors as require a mordant to develop or bring out the color are not fit for nor capable of use in food products.

Further, if the colored material is subjected to varying temperatures in the process of manufacturing foods, it should be able to withstand the effects of such temperatures, as, for example, in the manufacture of candies. The colors should also withstand the action of reducing agents, such as are generated in the course of fermentation and decomposition of the food product, or where a preservative such as sulphur dioxid is added to the food product to minimize the effect of decomposition of the food upon the color. Such colors are put on the European market, and perhaps, but not necessarily, on the United States market with preservatives added to them. Most of the coal-tar colors are susceptible to the action of sulphur dioxid, particularly when the latter has been used in the decolorizing of glucose, and Uranin (510) is one of the colors found to have the greatest resistance to the sulphur dioxid which may remain combined in candy.

For example, the book entitled "Henley's Twentieth Century Book of Receipts, Formulas and Processes," published in 1907, on page 359, says of sausage color:

> It is absolutely necessary in using aniline colors to add a disinfectant to the dyestuff solution, the object of which is, in case the sausage should commence to decompose, to prevent decomposition of azo-dyestuff by the disengaged hydrogen. Instead of boracic acid, formaline may be used as a disinfectant.

J. Fraenkel (*Arbeit. Kaiserl. Gesundh. 1902, v. 18, pp. 518–521;* abst. *Zts. Nahr. Genussm., 1902, v. 5, p. 986*) reports as follows on the composition of colors used in coloring sausages, meats, and preserves:

1. Blood color: Moisture, 15 per cent; common salt, 6.6 per cent; borax, 21 per cent; and Ponceau 2 R (G. T. 55).

2. Blood red for meat juices: Liquid of a specific gravity of 1.0163 not affected by acids or alkalis and containing 27 per cent total solids; of these total solids 31 per cent were salt, 12 per cent borax, and the remainder Ponceau 2 R (G. T. 55).
3. Casing red: This powder contained Orange II (G. T. 86).
4. Sausage red: A liquid containing Eosin.
5. Lobster color: A liquid of specific gravity 1.0064 containing 1.64 per cent of solids, of which 10.9 per cent were salt and the remainder Ponceau R T (G. T. 44).

G. Possetto (*Zts. Nahr. Unters. Hygiene Waarenk. 1891, v. 5, p. 105*) cited the following 15 colors as being used for the coloring of pastry:

	G. T. No.		G. T. No.
Martius Yellow	3	Tropæolin 00	88
Victoria Yellow	2	Tropæolin 000 No. 1	85
Naphthol Yellow S	4	Tropæolin 000 No. 2	86
Aurantia Yellow	6	Chrysoidin	17 18 41
Acid Yellow G	8		
Acid Yellow R	9		
Citronin	91	Azoflavin	92
Tropæolin 0	84		

Algerian Saffron (a mixture of Nos. 4 and 86 and crocein).
Prussian Saffron (composition not given).

"Blood-red" on the American market is starch colored with red coal-tar colors (*Ibid., 1896, v. 10, p. 114*).

"Butter yellow" is a clear saponifiable oil of reddish-yellow color, containing 3 per cent of anilin-azo-dimethylanilin (No. 16 of the Green Tables).

PROPORTION OF COAL-TAR COLOR USED.

The amount or proportion of coal-tar color used has been variously stated. On page IV of the Leffmann translation of Weyl's book entitled "The Sanitary Relations of the Coal-Tar Colors," it is stated that 1 ounce of Auramin (G. T. 425) will color 2,000 pounds of confectionery, which means 1 part of color in 32,000 parts of colored product.

Frentzel (*Zts. Nahr. Genussm., 1901, v. 4, pp. 968–974*), on authority not given, says that for sirups 1 part of color is used to from 4,000 to 5,000 parts of sirup; in colored sugars 1 part of coloring matter to from 1,333 to 4,000 parts of sugar; and in flour 1 part of coloring matter to from 666 to 1,000 parts of flour.

In pastry 1 to 100,000 parts (*Zts. Nahr. Unters. Hygiene, Waarenk. 1893, v. 7, p. 34*).

In chapter VII (p. 47), sections 15, 16, 17, and 18, are brought together statements made before the commission on regulations for the Federal food and drugs act, as to the amount of color contained in colored food products. Briefly these are as follows: Confectionery, 1 part of color in 3,500 parts of product; beverages 1 part of color in 128,000 parts, 256,000 parts, 1,024,000 parts; butter, 420 grains of color to 1,000 pounds of butter; or 1 part of color to 16,666 parts of butter.

One ounce of color to 30 pounds of "colored food;" the colored food was not further defined; which means 1 part of color in 480 parts of colored product.

It has further been represented that 1 part of color is sufficient to whiten 250,000 parts of yellow sugar.

From time to time others have presented information as to the amount of color used in food products. All of these data available have been tabulated, showing the number of parts of colored product containing 1 part of coal-tar color, arranged in the order of the amounts present:

Food [1]	100
Do [2]	480
Flour	666
Do	1,000
Sugar	1,333
Confectionery	3,500
Sirups	4,000
Sugar	4,000
Sirups	5,000
Confectionery	12,800
Butter	16,666
Confectionery	20,000
Confectionery	24,576
Do	30,000
Do	32,000
Beverages	80,000
Pastry	100,000
Beverages	120,000
Do	128,000
Confectionery	192,000
Whitening sugar	250,000
Beverages	256,000
Do	1,024,000

Grouped according to the kind of material colored, the ranges given are as follows:

Beverages	80,000; 120,000; 128,000; 256,000; 1,024,000
Butter	16,666
Confectionery	3,500; 12,800; 20,000; 24,576; 30,000; 32,000; 192,000
Flour	666; 1,000
Food	[1] 100; [2] 480
Pastry	100,000
Sugar	1,333; 4,000
Sirups	4,000; 5,000
Whitening sugar	250,000

These statements have emanated from persons presumably acquainted with the facts of their own practice, and if that presumption is correct it appears that there are wide variations in practice not only among individual users, but for individual colors. No attempt has been made to prove or disprove these statements by actual determination of the amount of color contained in commercial colored food products.

The 80 chemical individuals on this market for food-coloring purposes, it can be fairly assumed, have been tested and tried out as to their utility, and in this respect further tests were regarded as superfluous and therefore have not been undertaken.

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[1] Kind not definitely stated.

[2] Said to be for preserved tomatoes.

SUITABILITY OF SHADES OF PERMITTED COLORS AND MIXTURES OF SAME.

The shades produced by the seven permitted colors are, respectively, yellow, orange, blue, green, red, bluish scarlet, and brilliant cherry red. As statements are found in the literature against the use of all of the chemical individuals producing a brown or a violet shade, it will be necessary to produce these shades by a proper combination of two or more of the permitted colors. So far no criticism with regard to the shades produced by the seven colors themselves and by their appropriate mixture has been made that has been substantiated.

Objection has been made to the violet producible from blue and red, on the ground that when applied to a food product, such as candy, the component parts do not evenly fix themselves upon the material. This objection, however, has not been pressed and probably is not well taken, because of the fact, frequently reported, that few, if any, coal-tar colors are used without admixture of one or more other colors to shade or to tone the original color. This criticism, therefore, of the use of mixed colors can be regarded as not a serious objection.

One criticism urged with considerable persistency against the seven permitted colors was that none of them would withstand the action of the organic acids ordinarily found in beverages such as lemonade, and it was suggested that no color was proper for use for such purposes which would not withstand, unaltered, for a period of 12 hours the action of a 10 per cent solution of citric acid. In urging this objection substitutes were suggested for the permitted colors. The substitutes so urged were Tartrazin (94), Azorubin (103), Orange II (86), Ponceau 4 GB (13), and one other color designated as Scarlet SR, of whose chemical composition no information whatever was forthcoming. (The numbers in parentheses refer to the Green Tables.) Of the five colors suggested it can be said that concerning all but No. 103 adverse statements are found in the literature, and No. 86 is specifically regarded by every observer but one as being thoroughly poisonous. The suggested substitute list is, therefore, objectionable on the ground of injuriousness to health.

To test the validity of the assertion that none of the permitted colors could withstand the action of citric acid, solutions of the suggested colors, as well as of the permitted colors, each one in a thousand, were submitted to the action of citric acid, added in such quantity that it amounted to 10 per cent of the total bulk of solution. This experiment showed that Tartrazin is reddened by citric acid, whereas Naphthol Yellow S loses in tinctorial power to a slight extent. The shade produced by Ponceau 4GB can be closely imitated by a mixture of Naphthol Yellow S, Orange I, and Amaranth, all permitted colors. There is no choice whatever in the shade produced by the desired Azorubin and the permitted Amaranth, nor is there any difference in behavior toward citric acid. The difference in the shade between the desired Orange II and the permitted Orange I is so small that it requires a side-by-side comparison to distinguish between them. Moreover, the desired Orange II produces a precipitate when brought in contact with the citric acid, whereas the permitted Orange I does not so precipitate. The permitted Erythrosin is, of course, completely precipitated by the citric acid. The permitted Light Green and Indigo disulphoacid are weakened in tinctorial power by the addition of the citric acid. Of these colors the only ones used to any extent in beverages, so far as either the suggested or permitted list is concerned, are red, yellow, green, and orange.

As has been shown the permitted reds equal the desired reds and the permitted orange is better than the desired orange. The tinctorial power of the permitted yellow is not so great as the tinctorial power of the desired yellow, but this difference is so slight that the objection urged against the list of permitted colors, namely that they were so poor in quality that they had destroyed a profitable and lucrative business in the coloring of beverages, is untenable in view of the fact that, assuming a price of $1 per pound for Tartrazin, and 40 cents for Naphthol Yellow S, and using them in the proportions necessary to produce a lemonade color in a 10 per cent citric acid solution, it would take 5,000 quarts of finished lemonade to cause an increase of 1 cent in the cost of the production of the colored food product; that is, it increases the price per quart by one five-thousandth of a cent.

It has also been urged that the permitted green is not good enough for cordials and liqueurs, and that it is impossible to bring about the proper green by the use of the permitted yellow and blue. This criticism, however, has not been persisted in; the fact is that mixtures of the permitted yellow and blue can be made so as to obtain any desired shade of green, having a yellow or blue cast, and great clarity and brilliancy. How these mixed colors would look after a long period of time has not been ascertained.

It has also been said that the permitted red, Amaranth, is not a color suitable for the coloring of strawberry jams and it has been urged that the same chemical individual under another commercial name is better than the permitted red. This criticism has not been pressed, probably for the reason that it can not be substantiated.

Again it was claimed that the deposits to be noticed in bottled lemonades were due to Naphthol Yellow S, but solutions of Naphthol Yellow S in citric acid have remained without deposit for upward of 15 months; it is possible that such precipitation, if observed, may be due to an admixture of the nonpermitted Orange II with Naphthol

Yellow S, and this difficulty can be obviated by the use of the permitted Orange I, which does not precipitate in the citric acid, as shown.

Naphthol Yellow S has been objected to on account of the bitter taste it is said to impart to the beverages to which it is added, and steps have finally been taken by those interested to have another yellow placed on the permitted list.

It has also been objected that the permitted blue is not suitable for the coloring of sugar, first, because it is soluble, and second, because of the unsatisfactory shade. It may be sufficient, in answer to this criticism, to state that there was no insoluble blue offered on this market, and there was no blue other than the one permitted offered against which adverse statements did not exist in the literature, and in view of this state of affairs the criticism may be said to be not well taken.

As against all these specific criticisms it has been repeatedly stated by those in a position to know that they have found no difficulty whatever, by suitable mixtures of permitted colors, in reproducing any desired shade of any desired quality, not even excepting browns and violets.

Considering all of these criticisms, therefore, the conclusion seems reasonable that there is no serious or permanent objection to be made against the seven colors selected, either as to qualities for food-coloring purposes or range of producible shades.

IV. CONFORMITY OF FOOD-COLOR MARKET, 1907, TO RECOMMENDATIONS OF THE NATIONAL CONFECTIONERS' ASSOCIATION, 1899.[1]

Having thus shown that the food-color market of the United States contains not less than 80 coal-tar colors which are distinct chemical individuals, of which 74 are entered in the Green Tables and 6 are not, the next question to be considered is whether all of these substances are harmless and fit for use in food products.

As a guide in determining this point the "Official circular from the executive committee of the National Confectioners' Association of the United States," pertaining to colors in confectionery, dated February 1, 1899, may well be considered.

The function of this circular is said to be "to throw light upon the vexed question of what colors may be safely used in confectionery," evidently because "there may at times be a doubt in the mind of the honest confectioner as to which colors, flavors, or ingredients he may safely use and which he may reject."

The circular also states that "but infinitesimal amounts of color (coal-tar colors) need be or can be used to give the desired effects," and in view of this statement as to quantity it must be self-evident that a color harmful when used in the small quantities said to be used in confectionery is certainly harmful when used in the large quantities used in coloring other food products. This circular under the heading, "Colors that are injurious and therefore to be rejected—Harmful organic colors," enumerates 21 coal-tar colors. Of these 21 colors, 13, or 61.9 per cent, were among those submitted, and whose composition was stated by reference to Green Table numbers; the Green Table numbers of these colors, together with the number of sources from which they were obtained, follow:

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[1] See also p. 45.

Green Table numbers.	Number of sources.
11	2
17	2
18	1
84	2
86	8
95	2
106	5
169	1
197	4
201	2
398	2
584	1
650	2

Out of these 13 colors 3 each came from one source; 7 each came from two sources; 1 came from four sources; 1 came from five sources, and 1 came from eight sources, out of a possible 12; that is, one was wanted by more than half the sources.

It is further to be noted that of the trade names given to the submitted products of Class I the following appeared among the harmful list of this circular and also were found in identical form and spelling on the labels of the submitted products:

1. Bismarck Brown.
2. Chrysoidin R.
3. Chrysoidin Y.
4. Mandarin G extra.
5. Naphthol Green B.
6. Napthol Yellow.
7. New Coccine.
8. Orange A.
9. Orange A extra.
10. Orange G.
11. Orange II.
12. Scarlet.
13. Vesuvin B.
14. Crocein Scarlet 5 B.

The following parallel will serve to show the great resemblance between the names given to the harmful colors of the circular and those found on the samples submitted:

Circular's harmful list.	*Labels of submitted samples.*
Methylene Blue B B	Methylene Blue B.
Methylene Blue B B crystals	Methylene Blue D.
Methylene Blue B B G	Methylene Blue O.
Methylene Blue D B B	
New Coccine	New Coccine O Z.
	New Coccine Z.
Naphthol Green B	Naphthol Green.

Circular's harmful list.	*Labels of submitted samples.*
Orange II	Orange II Z.
Victoria Yellow	Victoria Yellow Conc. Z.
	Victoria Yellow Conc. T Z.
Acid Yellow	Acid Yellow G.
Bismarck Brown G	Bismarck Brown B.
Bismarck Brown T	Bismarck Brown B 216.
	Bismarck Brown Dark.
	Bismarck Brown R X.
	Bismarck Brown T D.
	Bismarck Brown Y Bril.
	Bismarck Brown Y Dark.
	Bismarck Brown 2 R X.
Chrysoin	Chrysoin Brown G Z.
	Chrysoin G E Z.
	Chrysoin R E Z.
	Chrysoin R Z.
Cochineal Red A	Cochineal Red.
Crocein Scarlet 3 B	Crocein Scarlet 10 B.
Crocein Scarlet 7 B	
Crocein Scarlet 8 B	
Fast Brown G	Fast Brown N.
	Fast Brown O.
Fast Yellow	Fast Yellow G.
	Fast Yellow O 3 3.
	Fast Yellow O 3 4.
	Fast Yellow Y.
Imperial Scarlet, in powder, extra	Imperial Scarlet 3 B.
Safranin	Safranin S P.
Safranin A G extra.	
Safranin AGT extra.	
Safranin Conc.	
Safranin extra G.	
Safranin FF extra No. 0.	
Safranin G extra GGS.	
Safranin G 000.	
Safranin T.	

This comparison disclosed a considerable lack of conformity between the United States food-color trade in 1907 and the circular of February, 1899, upon whose preparation for seven months prior to its date "a great deal of thought and labor have been given to a thorough investigation of the whole subject of 'colors in confectionery,' in which the committee has been largely aided by the researches of the association's chemist and by the results of his analytical tests" and whose "classifications have been carefully made, and are based upon the authority of the eminent chemists, Prof. Koenig and Prof. Weyl, upon the resolutions of the Swiss chemists and upon the French ordinances regarding the coloring of food products," and which list was expected to be "of value to color dealers and chemists," and also was published to assist the confectioner in obeying "the letter and the spirit" of the pure-candy laws.

Under the heading "Colors that have been shown to be harmless as used in the confectioner's art, harmless organic colors," this circular enumerates 36 colors, for 4 of which there are no Green Table numbers. Of the 32 colors having Green Table numbers, 20, or 62.5 per cent, were among those colors submitted. The Green Table numbers of these colors, together with the number of sources from which they were obtained, follow:

Green Table numbers.	Number of sources.	Green Table numbers.	Number of sources.
4	10	240	1
8	5	269	1
9	1	287	1
13	6	427	2
55	5	448	4
65	2	451	5
85	2	462	2
103	6	512	3
105	1	517	5
107	7	520	2

Of these 20 colors 5 each came from 1 source; 5 each came from 2 sources; 1 came from 3 sources; 1 came from 4 sources; 4 each came from 5 sources; 2 each came from 6 sources; 1 came from 7 sources, and 1 came from 10 sources; that is, only 2 were wanted by more than half the makers or importers, and only 4 by half the sources.

It will be noted that this circular provides for a total of only 57 different coal-tar colors; the number of avowed colors submitted reached 80, or 23 in excess of this number, and further, that out of the 57 colors referred to in this circular only 33 appeared among those colors submitted whose composition was acknowledged, so that for 47, or 58.8 per cent of the avowed submitted colors, this circular is no specific guide. From the data obtainable from this circular, the following tabulation can be made:

Harmfulness of submitted colors based on list in circular.

Data.	Total on market.	Disclosed colors on United States market.	Circular's corresponding list.
		Per cent.	*Per cent.*
Harmful	13	16.3	61.9
Harmless	20	25.4	62.5

These figures disclose a considerable and self-evident disregard of the request, then eight years old, as made by the National Confectioners'

Association of the United States, that coal-tar colors designated by it as "colors that are injurious and therefore to be rejected, harmful organic colors" be not used in confectionery, and by implication that they should not be used in other food products.

In view of this disregard with respect to 13 coal-tar colors out of 33 on the United States market in the summer of 1907, dealt with in this confectioners' list, the conclusion seemed justified that the action regarding other coal-tar colors on the United States market in the summer of 1907 was equally heedless or indifferent, a conclusion which is borne out by the material brought together under section IX.

The necessity of adhering to some unequivocal terminology, as has been done in these pages by referring to the serial numbers in the Green Tables, appears from the following:

Trade names are not definite with respect to the composition of the article sold under a given name, although tinctorially the colors may be substantially equivalent. In the Green Tables there are not less than 29 instances where the same trade name is applied to two or more different chemical individuals. In some cases these chemical individuals are fairly closely related, in others they are only remotely related. When the differences are greater than the presence or absence of a sulpho group or the use of methyl for ethyl or the reverse, the instances are given below:

1. Cotton Yellow.........	128	Primulin-azo-m-phenylene-diamin-disulphonic acid.
	191	Diphenylurea-disazo-bi-salicylic acid.
2. Methyl Eosin..........	513	Methylated tetrabromo-fluorescein.
	375	Dinitro dibromo fluorescein.
3. New Yellow..........	88	p-sulphanilic acid azo-diphenylamin.
	91	Nitration product of diphenylamin yellow.
4. Orange III...........	23	Meta-nitranilin azo-R salt.
	87	p-sulphanilic acid azo-dimethylanilin.
5. Orange N.............	43	Toluidin azo-Schaffer acid.
	88	p-sulphanilic acid azo-diphenylamin.
6. Orange R.............	97	o-toluidin-monosulphonic acid azo-betanaphthol.
	15	Anilin-azo-R salt.
	99	Xylidin sulphoacid-azo-betanaphthol.
7. Toluylene Red.......	261	Dichloro-benzidin disazo R salt.
	580	Dimethyl diamido toluphenazin.

The following quotation also bears on this point:

> In attempts to group the aniline colors a kind of uncertainty appears even among color chemists. The same trade name does not always correspond to the same preparation. Many preparations are not chemical individuals, but mixtures of related colors. Many preparations are "standardized" for the trade; for example, with dextrin. On account of the patent laws, factory secrets surround the production of many coloring matters, and frequently statements are met with which are directly intended for the purpose of misleading competition. *(Hueppe, Die Methoden der Bakterienforschung, 5th ed., 1891, p. 105.)*

V. SOME LEGAL ENACTMENTS RELATIVE TO THE USE OF COAL-TAR DYES.

LIST OF THIRTEEN FOREIGN LEGAL ENACTMENTS.

The 13 foreign legal enactments compared are as follows:

1. The Austrian regulation of March 1, 1886, which forbids No. 483 of the Green Tables and all anilin colors.
2. The law of Austria of May 1, 1886, which forbids No. 1 of the Green Tables and all anilin colors.
3. The Austrian regulation of September 19, 1895, in which certain colors only are permitted, and all others are forbidden. There are 16 titles of permitted colors in this law, but these are in some cases so elastic and so indefinite as to include 47 entries in the Green Tables.
4. The Austrian law of January 22, 1896, in which there are 17 titles, but these are sufficiently elastic to allow of 22 entries in the Green Tables being included in them.
5. The German law of July 5, 1887, which specifically prohibits only Nos. 1 and 483 of the Green Tables. The interpretation which seems to be generally placed upon this law is that all other Green Table members are permitted in Germany for use in foods.
6. The Italian law of February 7, 1892, forbidding all colors except 9 different titles, which, however, were elastic enough to include 32 entries in the Green Tables.
7. The law of Italy of February 7, 1902, which prohibits 37 entries in the Green Tables, and permits 11 specifically.
8. The Italian decree of June 29, 1893, in which there were 7 titles of permitted colors sufficiently elastic to include 34 different individuals.
9. The Italian decree of March 24, 1895, forbidding four titles covering only four entries in the Green Tables.
10. The French police ordinance of May 21, 1885, in which 489 entries in the Green Tables were prohibited.
11. The French police ordinance of December 31, 1890, in which 469 entries of the Green Tables were prohibited, and which also permitted under 9 titles 23 entries in the Green Tables.
12. The Belgian law of 1891, which specifically forbids only four entries in the Green Tables.
13. The law of the Canton of Tessin, dated May 18, 1897, which forbids only 4 specific entries in the Green Tables.

SUMMARY OF COLORS PERMITTED BY THESE LEGAL ENACTMENTS.

An examination of the 13 legal enactments made in Europe with respect to the use of coal-tar colors in food products discloses considerable difference of opinion as to the harmfulness or the harmlessness of even the same chemical individuals. To prepare an approximate and comprehensive summary of the effect of such legislation, the following plan has been followed:

The provisions of a selected number of laws and regulations, 13 in number and dating from 1883 to 1902, were read with respect to permission or prohibition of the 695 chemical individuals listed in the Green Tables. The effect of each law upon each separate entry in the Green Table numbers was noted, either as permitted, forbidden, or noncommittal when the law was silent upon such entry; the laws were read with the understanding that what was not forbidden was permitted, and what was neither forbidden nor permitted was noncommittal; if this were an incorrect or improper procedure the number of permitted colors would be reduced but not augmented. For the purposes of this first approximation, no attempt was made to segregate those specifically permitted from those permitted by blanket expressions or phrases.

For the purposes of a side-by-side comparison, the term "index number" was coined; this "index number" gives in the first place the number of legal enactments that permit the color; in the second place the number of enactments that forbid it; and in the third place the number of enactments that are silent or noncommittal. Thus: 283 as an index number would mean 2 enactments permit, 8 forbid, and 3 are noncommittal; so that an index number with the highest hundreds would have the greatest number of permissions, and with the highest tens would have the highest number of prohibitions. With this understanding of these terms, the following table of so-called index or "P. F. N." numbers is offered:

Thirteen legal enactments classified by Green Table numbers and the "P. F. N. figure," or "index number."

Total number of Green Table entries.	Index number.	Green Table numbers.
1	184	483.
1	274	534.
3	2,10,1	1–3.
217	364	394–411, 415–426, 429–434, *435*[1], 436–445, 484–492, 528–561, 564–583, 585–598, 600, 602–649, 651–691, 693–695.
1	373	103.
366	382	6, 7, 10, 11, 12, 14, 16, 19–22, 35–40, 42, 43, 45–54, 58–64, 66–83, 89, 90, 93, 94, 96–101, 104–106, 109–113, 115–145, 149, 151–156, 158, 159, 161, 162, 164–168, 172, 197, 199–243, 245–393, 412–414, 493–511, 524–527, 650.
29	391	446, 447, 449, 450, 453, 455, 456, 458–461, 463–476, 478, 479, 481, 482.
3	454	428, 563, 599.
1	463	*692*.
22	472	5, 8, 9, 23–34, 84, 86–88, 91, 520, 523.
7	481	95, 452, 454, 477, 513, 519, 522.
2	544	562, 601.
2	562	515, 516.
3	571	480, 514, 521.
3	643	44, 114, 427.
11	652	13, 15, *85*, 92, 108, 146–148, 150, 160, *517*.
6	661	17, 18, 41, 169, 512, 518.
2	670	451, 462.
6	742	*4*, 55, *56*, 57, 102, 244.
6	751	65, *107*, 163, 170, 198, 448.
1	850	457.
2	931	157, 171.

[1] Italicized figures indicate colors permitted by F. I. D. 76.

From the table it appears that there is no one Green Table number that is permitted by each and all of these 13 legal enactments, nor is there any one color that is prohibited by each and all of these 13 legal enactments; and as late as 1902 there was considerable confusion as to what should or should not be permitted or forbidden. That the European enactments were not in reality consistent or effective appears from the following:

1. In respect to the use of coal-tar colors, the views as to their harmfulness or harmlessness are very divergent, and this uncertainty is expressed in the various legislative enactments. (v. Raumer, *Zts. Nahr. Unters. Hygiene & Waarenk., 1895, v. 9, p. 207.*)

2. After eating groats, which no doubt were free from ordinary poisons but had been colored with Martius Yellow, a whole family became sick. Since this coloring matter is not mentioned among those which, under the law of July 5, 1887, are forbidden for the purpose of coloring articles of food, a complaint could not be lodged. Nevertheless, the use of Martius Yellow for the coloring of articles of food would seem to be dangerous, since this coloring matter exerts poisonous effects. It is a weakness in the law that coal-tar colors, of which new members are continuously appearing on the market, and whose physiological action is unknown, should be at all permitted for the coloring of articles of food. (Dietrich, Th., *Jahresberichte der landwirthschaftlichen Versuchsstation, Marburg, 1900–1901, p. 13;* abst. *Zts. Nahr. Genussm., 1902, v. 5, p. 364.*)

On account of their large number and the great diversity of opinion as to harmfulness of some coal-tar colors and the harmlessness of others therein reflected, no attempt was made to collect all the legal enactments and regulations made with respect to coal-tar dyes. The foregoing are typical of the remainder.

COLORS SAID TO BE PERMITTED UNDER THE GERMAN LAW OF 1887.

That some of the laws did not employ specific terms but used those possessed of a great degree of elasticity appears from the following taken from Weyl's "Sanitary relations of the coal-tar colors," page 38, concerning the Chamber of Commerce and Trade of Sonneberg which declared on December 4, 1887, that the German law of July 5, 1887, allowed the unrestricted use of—

> All blue and violet anilin (that is coal-tar) colors, all ponceaus, all orange colors, Methyl Green, Brilliant Green, Malachite Green, Chrysoidin, Naphthol Yellow, Martius Yellow, Eosin, Phloxin, Safranin, Erythrosin, Fuchsin, Phenylene Brown, and Anilin Black.

This amounts to not less than 233 permitted colors, as will now be shown.

Using the Green Tables as a guide it will be found that under the above ruling there are to-day no less than 107 blue coal-tar colors which could be used for food coloring. They are arranged as follows, showing the comments on same in the literature.

BLUE COLORS.

Unfavorable: 478, 479, 488, 490, 572, 602, 639. (Total, 7.)

Favorable: 477, 599, 600, 692. (Total, 4.)

Contradictory: 287, 457, 480, 563, 601, 650, 689. (Total, 7.)

Not reported on: 36, 83, 119, 142, 175, 189, 209, 246, 247, 254, 257, 263, 266, 288, 290, 291, 292, 293, 294, 295, 301, 302, 310, 311, 314, 315, 316, 317, 318, 319, 323, 327, 345, 347, 348, 351, 352, 356, 359, 364, 430, 432, 439, 440, 442, 444, 456, 473, 476, 481, 482, 487, 492, 509, 542, 551, 556, 558, 559, 560, 562, 569, 595, 598, 608, 611, 612, 615, 618, 619, 621, 625, 627, 628, 634, 637, 638, 640, 641, 642, 643, 652, 655, 656, 657, 664, 682, 693, 694. (Total, 89.)

Similarly there would be 50 violets which are classified as follows:

VIOLET COLORS.

Unfavorable: 620, 649. (Total, 2.)

Favorable: 467, 593. (Total, 2.)

Contradictory: 450, 451. (Total, 2.)

Not reported on: 30, 34, 36, 118, 176, 179, 207, 244, 246, 248, 252, 256, 273, 320, 336, 338, 340, 342, 343, 444, 452, 454, 455, 463, 464, 465, 466, 468, 469, 470, 471, 472, 474, 486, 506, 507, 525, 552, 579, 581, 585, 592, 613, 625. (Total, 44.)

Similarly there would be 18 ponceaus which are classified as follows:

PONCEAUS.

Unfavorable: None.

Favorable: 169, 448. (Total, 2.)

Contradictory: 13, 15, 55, 160, 163. (Total, 5.)

Not reported on: 44, 56[1], 57[1], 108, 113, 114, 146, 147, 148, 150, 165. (Total, 11.)

Similarly there would be 35 Oranges which are classified as follows:

ORANGES.

Unfavorable: 2, 97. (Total, 2.)

Favorable: 85. (Total, 1.)

Contradictory: 14, 18, 43, 86, 87, 88, 95. (Total, 7.)

Not reported on: 10, 23, 47, 54, 99, 100, 136, 162, 196, 217, 218, 222, 225, 235, 236, 265, 275, 392, 406, 408, 409, 529, 531, 545, 547. (Total, 25.)

The remaining 23 colors named are classified as follows:

MISCELLANEOUS.

Unfavorable: Martius Yellow (3).[2] (Total, 1.)

Favorable: Naphthol Yellow S. (4); Eosin (512, 517, 521); Phloxin (521); Erythrosin (517). (Total, 6.)

Contradictory: Brilliant Green (428); Malachite Green (427); Chrysoidin (17, 18, 41); Safranin (584); Fuchsin (448); Phenylene Brown (197). (Total, 8.)

Not reported on: Methyl Green (460, 461); Eosin (514, 515); Phloxin (518); Safranin (583, 585); Erythrosin (516); Anilin Black (577). (Total, 9.)

It will be noticed that names such as Eosin, Erythrosin, and Phloxin appear in more than one classification; thus some of each are favorably reported on and the others are not reported on at all.

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[1] Nos. 56 and 57 are included among the permitted colors of the Austrian law.

[2] The numbers in parentheses following the names are the Green Table numbers.

The 233 colors said by the Sonneberg Chamber of Commerce and Trade to be entitled to unrestricted use in food coloring under the German law of 1887 referred to may also be classified as follows:

Summary of classification of colors permitted under the German law of 1887 according to comments in the literature.

Class.	Total.	Comments in the literature.			
		Unfavorable.	Favorable.	Contradictory.	Not reported on.
Blues	107	7	4	7	89
Violets	50	2	2	2	44
Ponceaus	18	0	2	5	11
Oranges	35	2	1	7	25
Miscellaneous	23	1	5	8	9
Total	233	12	14	29	178

Out of these 233 colors only 55, or about one-fourth, have been reported on in the literature and the remaining three-fourths have not been examined at all. To the 55 examined and reported on there may be added 2, namely, Nos. 56 and 57, since they are included among the colors once permitted by law in Austria, thus making a total of 57 examined out of 232. Adding these two to the 14 favorably reported on makes a total of 16. It finally appears that 41 out of 57 colors examined would in the light of present knowledge be improper to be used in food and 12 at least should not be used for such purposes at all.

The classification into unfavorable, favorable, contradictory, and not reported on is based upon the tabulation on page 63; if that is substantially correct the above conclusions are also true.

The foregoing side-by-side comparison of 13 legal enactments, while it makes no claim to being absolutely and wholly accurate in all the classifications or conclusions drawn, is no doubt a fair reflection of the condition of mind of those framing the enactments, and consequently of information upon which those enactments were based; and the conclusion would therefore seem to be justified that the fact that a color has been permitted or has been forbidden by any one or the majority of these legal enactments ought not to constitute a clean bill of health, nor an indictment, as the case may be.

This side-by-side comparison must not be pushed to extremes; indeed the extent to which it can be employed is naturally very limited, and the purpose for which it was made was to reflect in a manner easily and comprehensively grasped the confusion and inconsistencies which very persistently force themselves upon the mind of a person reading those enactments and having in mind the chemical individuals at which they are aimed.

The definite lesson to be learned from this side-by-side comparison is that these enactments in many cases employed terms so vague and indefinite as to permit the use of some bad colors as well as all good ones, that is they were not sufficiently definite to exclude all that were harmful.

DEFINITENESS AND DETAIL NECESSARY TO EFFECT QUALITY CONTROL.

This apparent state of confusion in legal enactments that preceded the summer of 1907 was a very strong factor in the formation of the conclusion that in order to be effective any law or regulation dealing with coal-tar colors for use in foods must prohibit every coal-tar color except certain definite specific ones.

The Austrian laws of September 19, 1895, and of January 22, 1896, provided for quality control by public and other laboratories of the coal-tar colors put upon the market for use in foods; the results of such control, as reported in the Zeitschrift für Nahrungsmittel-Untersuchung, Hygiene und Waarenkunde, 1896, v. 10, p. 335, are as follows:

> Coloring matters of commerce are mostly mixtures of various coloring matters, a right which manufacturers will not part with; and further, while it is indeed possible to test the coloring matter in substance, it is nevertheless impossible to test it in the very small amounts which are used in the coloring of foodstuffs and to determine with certainty the identity of the color as to whether it is or not one of the permitted colors.
>
> Of 21 samples of coloring matter examined, 14 were objectionable, partly because of false labeling, or because they were mixtures, partly because they contained poisonous metals, or a forbidden coloring matter. Thus, a so-called "Evergreen" was Naphthol Green B, a poisonous nitroso color; Malachite Green contained zinc; an Acid Magenta and a Rosalin contained traces of copper; Ponceau, Eosin, Brown, and Roccellin contained traces of tin; Orange I and Waterblue contained traces of tin and zinc. The last-named coloring matters were therefore not prepared in proper state of purity.
>
> The authorities in Vienna examined four and rejected two colors. (*Ibid., 1898, p. 107.*)

The Swiss authorities exercised control over colors, after they reached the market, with the result that the authorities in Basle examined ten colors and rejected one. (*Ibid., 1897, p. 292.*)

These facts, together with the knowledge derived by even the most superficial ocular examination of the 294 specimens received in the summer of 1907, played a very great part in the formation of the conclusion that control of quality, in order to be even reasonably effective, must be thoroughgoing, and that colors must be excluded from the market until they prove themselves to be clean, rather than permitted promiscuously and then driven out of the market by the authorities if unsuitable.

The effective quality control of food colors requires careful and searching examination of a kind which can not usually be obtained by the general purchasing public. The quality of the food colors offered in the summer of 1907 varied greatly, and the substances contaminating them were of such indefinite and probably variable composition (of whose physiological action nothing definite was known and whose quick and certain detection in the colored food product would be very complicated, if not impossible) that quality control of greater efficiency than that exercised by those selling food colors in the summer of 1907 seemed necessary on the part of the authorities having charge of the enforcement of the food and drugs act.

The points of original entry of food colors into the United States food-color market are relatively few, whereas the points of distribution of food colors are very many, the former being less than 20 and probably fewer than 10, while the latter may number up into the hundreds; therefore, not only is the labor and the expense of quality control of food colors reduced to its probable minimum by keeping food colors off the market until they have shown their right to be so used, but also the certainty and the efficiency of quality control is increased to its probable maximum.

The quality control thus suggested is similar to that exercised by the States of New York, Michigan, and Ohio over salt before it enters the market for human consumption. The method of color control here suggested differs only in degree, not in kind, from the quality control exercised over salt by the States named. Experience has shown such quality control of food colors to be not only practicable but capable of realization without any hardship and but little, if any, inconvenience to those concerned.

STATE LAWS PROHIBITING THE USE OF COLORS IN CERTAIN FOODS, 1909.

The laws of the individual States of the United States have also restricted the use of coal-tar coloring matters in foods. These restrictions are directed principally against the use of color to conceal inferiority, which restriction is found in almost all the States.

The sale of poisonous coloring matters for foods is prohibited in the State of New York, and in New York and North Carolina the addition of injurious colors to foods is prohibited.

Minnesota and North Carolina prohibit coal-tar dyes in all foods.

Foods and beverages are considered adulterated in North Dakota and Wyoming if they contain aniline dyes or other coal-tar dyes.

Artificial coloring is prohibited in sausages by Colorado and Wisconsin.

Artificial coloring, including, of course, coal-tar colors, must not be added to vinegar in the States of Arkansas, California, Connecticut, Iowa, Minnesota, Missouri, New Jersey, New York, Pennsylvania, Tennessee, Wisconsin, and Wyoming.

Distilled vinegar must not contain artificial color in Ohio and Oklahoma, and must be free from harmful artificial coloring matter in Utah.

In South Dakota oleomargarine must not be colored.

Artificial coloring is prohibited in milk by California, Oklahoma, Pennsylvania, Utah, and Wisconsin and in cream by California Connecticut, Pennsylvania, Utah, and Wisconsin.

Coal-tar dyes are inhibited in cakes, crackers, candy, ice cream, and like products by Virginia. Ice cream is considered adulterated in Michigan if it contains harmful colors.

Forty-six States prohibit the use of poisonous colors in candy. They are as follows: Alabama, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Philippine Islands, Porto Rico, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, Wisconsin, and Wyoming.

VI. RECOMMENDATIONS BY ASSOCIATIONS AND INDIVIDUALS AS TO USE OF COAL-TAR DYES AS FOOD COLORS.

CAZENEUVE AND LÉPINE.

Cazeneuve and Lépine (*Bull. de l'acad. de médicine, April 27, 1886, p. 643*) says:

> We have arrived at the following conclusions:
>
> 1. The nitro derivatives are especially poisonous (dinitronaphthol being comparable with picric acid), but the sulphonated product is harmless.
>
> 2. Safranin and Methylene Blue are harmful, producing gastric intestinal disturbances, being violent poisons.
>
> 3. The following coloring matters are tolerated by man, whether well or affected with Bright's disease; similarly, too, animals (dogs, guinea pigs) without any noticeable disturbances and at rather high doses:

	Probable Green Table Nos.
1. Fast Yellow	9
2. Roccellin	102
3. Bordeaux B	65
4. Purple	106 or 107
5. Ponceau R	55
6. Orange I	85
7. Fuchsin S	462

> Among the nontoxic sulphonated colors we have been able to make out the following list or classification, based upon their power of producing disturbances, proceeding from the least inert to the most inert:

	Probable Green Table Nos.
1. Orange I	85
2. Bordeaux B	65
3. Ponceau R	55
4. Roccellin	102
5. Yellow NS	4
6. Fast Yellow	9
7. Purple	106 or 107

The results of our experiments have led us to the following conclusions:

1. The relative nonpoisonous nature of the azo colors used for coloring wines explains why this artificial coloring has not caused any real epidemic.

2. This artificial coloring of wines by coal-tar colors is dangerous. It opens the door to the employment of coloring matters of very variable and noxious properties. Thus Martius Yellow, which is poisonous, has been used for 10 years past to color pastry (3 grams per 100 kilograms) and it may be used to-morrow, perhaps, to color wines mixed with a red or a blue.

3. A rigid law against the artificial coloring of wines ought to be promulgated, particularly if this coloration covers detestable practices most prejudicial to the public health. The addition of salicylic acid, glycerin, and tartaric acid, or the acidifying by sulphuric acid, is cloaked by the use of the coloring matter.

Some sulphonated azo-coloring matters are sufficiently inert to enable their being employed as artificial color in foods, bonbons, and liquors. These colors are manufactured according to simple processes which give theoretical yields and no metallic salt, such as mercury, tin, or arsenic participates; sulphate of soda is the only impurity.

In view of the great extent of the use of these coloring matters, it is better to regulate their consumption by tolerating certain of these products rather than to interpose an illusory barrier to their use. Where you can not arrest a stream you can at least regulate its course.

It would be better definitely to classify these substances with respect to their noxious properties, tolerate some and prohibit the others, rather than to be exposed to the consequences of permitting manufacturers to introduce into food, without any scientific control whatever, any products whatever.

These coloring matters should be sold in commerce under the names of harmless colors as determined by analysis. By chemical analysis it would be recognized as to whether we were dealing with one color or with a mixture of two or three colors.

The colors most used are made up of red, yellow, and blue, which apparently imitate the appearance of the wines of the Midi. Thus we have recognized such coloring matters in Roccellin, Naphthol Yellow, and Methylene Blue.

Sulphonated Fuchsin is very much used, combined with a yellow and a blue. This mixture turns green with ammonia, like the coloring matter of wine. In fact, Acid Fuchsin is decolorized by ammonia. The yellow and blue remain intact, and give a green which suggests true wine color.

SOCIETY OF SWISS ANALYTICAL CHEMISTS.

In 1891 the Society of Swiss Analytical Chemists recommended that certain coloring matters which are to be regarded as harmful to health should not be permitted to be used in the preparation of articles of food intended for sale in which artificial coloring is at all permitted.

The coal-tar colors thus prohibited are identified in the following by their Green Table numbers, only one trade name being given: Picric Acid (1); Dinitrocresol (2); Martius Yellow (3); Aurantia (6); Orange II (86); Metanil Yellow (95); Safranin (584); Methylene Blue (650). (*Zts. Nahr. Unters. Hygiene, 1891, v. 5, p. 293.*)

TSCHIRCH.

In 1893 Tschirch recommended as follows:

1. The coal-tar colors, and in a narrow sense the anilin colors, are no longer harmful on account of their arsenic content, since at the present time the great majority of them are prepared free from arsenic.

2. Some colors have shown themselves to be harmful to the system.

3. Coal-tar colors in general should therefore be permitted for the coloring of foods, but those that have been found to be harmful should be expressly and specifically forbidden.

4. The amount of coloring matter which has been determined quantitatively in bonbons and liqueurs is so small that even the ones regarded as poisonous would not be able to develop their harmful effects. (*Zts. Nahr. Unters. Hygiene Waarenk., 1893, v. 7, p. 338.*)

KAYSER.

In 1895 Kayser expressed himself as follows:

As to the poisonous nature of organic coloring, and in respect to their composition, H. Erdmann (*Pharm. Centralh., 1892, v. 33, p. 357*) concludes that in general acid dyestuffs can pass as nonpoisonous; whereas in the case of basic coloring matters it is recommended to make a physiological examination before using them for the coloring of things in daily use, especially articles of food. Whether that portion of that view which deals with acid dyestuffs will retain unexceptionable and positive validity appears doubtful.

At the present time, speaking generally, interested manufacturers take the point of view that all the coloring matters which are not forbidden in the food law are to be regarded as permitted. Whether this point of view is free from legal objection can not be discussed here; that, however, it can not be accepted from a hygienic point of view under any circumstances whatever does not require any special proof for those conversant with the facts. The hygienic requirements under all circumstances can be summed up in the following rule:

Every coloring matter is to be regarded in every way as suspicious, so far as its harmlessness is not proven by experience or by correct physiological experiments.

No one can say in advance that among the colors which are to-day manufactured and used, which are as yet not called into question, there are none which possess distinctly poisonous properties. Correct examination of artificial coloring matters in this direction is, as is well known, even to the present almost wholly lacking. (*Forschungsberichte über Lebensmittel*, etc., *1895, Vol. II, p. 181.*)

WEYL.

In 1896 Weyl expressed himself as follows:

Since the number of the organic coloring matters already known is a very large one, and since their number is increasing daily, and it seems to be unlikely that each individual of these coloring matters will be examined as to its poisonous nature, there are only two ways left in which to solve the question as to the use of coloring matters in the manufacture of food and articles of daily use.

One of them, and at the same time the simplest, would be to prohibit the use of all coloring matters for the coloring of foods, etc. This rigorous point of view will hardly ever be taken by legislators, because it would be tantamount to the removal of many marks of differentiation which have become desirable and necessary.

The second way seems to be the much more practicable, and which Theodor Weyl proposed some time ago.

It consists in permitting the use of only a definite number of coloring matters, recognized as harmless, for the coloring of articles of food, etc. Which coloring matters are to be so permitted is to be determined by the authorities having jurisdiction. The same authorities are also to determine the maximum amount of each coloring matter which can be used for any purpose. New coloring matters can be used only for the above-mentioned purposes when they have been recognized as nonpoisonous after official test. All permitted coloring matters must be also detectable, even in small amounts. (*Handbuch der Hygiene, 1896, Vol. III, p. 385.*)

NATIONAL CONFECTIONERS' ASSOCIATION.

In 1899 the National Confectioners' Association of the United States issued an official circular, which has been previously discussed,[1] designating certain colors as harmful, and certain others as harmless; the members of each class are given in the following list, in which only one trade name is given, the Green Table number appearing in parentheses at the end of that name.

HARMFUL ORGANIC COLORS.

Red colors: Ponceau 3RB (163); Crocein Scarlet 3B (160); Cochineal Red A (106); Crocein Scarlet 7B (169); Crocein Scarlet O extra (164); Safranin (584).

Yellow colors: Picric Acid (1); Martius Yellow (3); Acme Yellow (84); Victoria Yellow (2); Orange II (86); Metanil Yellow (95); Sudan I (11); Orange IV (88).

Green colors: Naphthol Green B (398).

Blue colors: Methylene Blue BBG (650).

Brown colors: Bismarck Brown (197); Vesuvin B (201); Fast Brown G (138); Chrysoidin (17, 18).

HARMLESS ORGANIC COLORS.

Red colors: Artificial Alizarin and Purpurin (534); Eosin (512); Erythrosin (517); Rose Bengale (520); Phloxin (521); Ponceau 2R (55); Bordeaux B (65); Ponceau 2G (15); Fuchsin S (462); Archil Substitute (28); Orange I (85); Congo Red (240); Azorubin S (103); Fast Red D (107); Fast Red (105); Ponceau 4GB (13); Fuchsin (448).

Yellow and Orange colors: Naphthol Yellow S (4); Brilliant Yellow (5); Fast Yellow (8); Fast Yellow R (9); Azarin S (70); Orange (43).

Green colors: Malachite Green (427); Dinitrosoresorcin (394).

Blue colors: Indigo (689); Gentian Blue (457); Coupiers Blue (600).

Violet colors: Paris Violet (451); Wool Black (166); Azoblue (287); Mauvein (593).

Brown colors: Chrysamin R (269).

SCHACHERL.

Schacherl in 1903 made the following statement:

> If coal-tar colors are to be permitted for the coloring of food, then, in my opinion, it is not right to limit the use of such to a few coloring matters, but groups of coloring matters must be permitted which are without suspicion from a sanitary standpoint, and which are characterized by definite reactions. Other groups, on the other hand, which contain harmful or merely suspicious colors, must be absolutely excluded. * * * The selection would be easily made if sufficient data were at hand with respect to the physiological action. Unfortunately this is lacking, a circumstance which need not be surprising in view of the very large number of synthetic coloring matters, since the Schultz-Julius tables enumerate 681 such colors. Unfortunately the experiments of Th. Weyl, which were planned on a large scale, have not been completed, and apart from isolated investigations we are limited in passing judgment upon the most of these colors to the proof that to date nothing with respect to harmful action has become known. * * * Consequently it is still possible that one or the other coloring matter which may to-day be regarded as above suspicion, or a newly discovered coloring matter may possess poisonous properties; the legislators should under all circumstances have the power to exclude for use in foods all suspicious combinations, and all such coloring matters as are not easily distinguishable from them.

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[1] See also p. 30.

The final recommendations of Schacherl amount to permitting—

1. All the Azo colors, Nos. 7 to 393 of the Green Tables, except No. 86.
2. All the Triphenylmethane colors, Nos. 427 to 492 of the Green Tables, except the hydroxyl derivatives, which would be Green Tables Nos. 483, 484, 485, 486, and 491.
3. All Pyronins, Nos. 493 to 527 of the Green Tables.
4. All Oxyketones, Nos. 537 to 570 of the Green Tables.
5. All Indulins, Nos. 599, 601, and 603 of the Green Tables.
6. Naphthol Yellow S, G. T. No. 4.
7. Methylene Blue, G. T. No. 650.

The use of all other coal-tar colors would best be forbidden, partly from hygienic and partly from practical considerations (rendering control more easy), until the absolute harmlessness of the group in question is determined by physiological test. * * * It should be required of all permitted coloring matters that they shall not contain substances which are harmful to health, or even suspicious, either in chemical union or as contaminations. (*Fifth International Congress of Applied Chemistry, Berlin, 1903, Vol. IV, pp. 1041–1048.*)

The exclusions recommended are all nitro-colors, except Naphthol Yellow S; all acridin colors and all chinolin colors; Auramin, Indophenol and all nitro-colors, except Naphthol Yellow S, Schacherl regards as not necessary; further, he has no knowledge of the physiological action of any of the azoxy or the thiobenzenyl colors, and aside from Methylene Blue, he has no knowledge of the physiological action of the oxazins and thiazins.

CLASSIFICATION OF RECOMMENDATIONS IN THE LITERATURE.

The following table shows the groups of coal-tar colors of the Green Table classification and the Green Table numbers of the members of each of the groups, together with the favorable or unfavorable recommendations found in the literature in regard to each and a statement as to those regarding which no recommendations are made:

Tabulation of recommendations found in the literature.

Group number.	Green Table number.	Name of color.	Number of individuals reported on—			Total number.	
			Unfavorably. (1)	Favorably. (2)	Conflictingly. (3)	Reported. (4)	Not reported. (5)
1	1–6	Nitro	4	2	0	6	0
2	7–132	Monoazo	3	10	19	32	94
3	133–336	Disazo	3	3	7	13	191
4	337–382	Trisazo	....	....	....	....	46
5	383–393	Tetrakisazo	....	....	....	....	11
6	394–398	Nitroso	0	1	1	2	3
7	399–416	Stilbene	0	1	0	1	17
8	417–424	Oxyketone	....	....	....	....	8
9	425–426	Diphenylmethane	1	0	0	1	1
10	427–492	Triphenylmethane	5	5	8	18	48
11	493–527	Xanthenes	2	5	1	8	27
12	528–533	Acridin	2	0	0	2	4
13	534–570	Anthracene	0	0	1	1	36
14	571–578	Indophenols	2	1	0	3	5
15	579–616	Azins	2	3	2	7	31
16	617–648	Oxazins	2	0	0	2	30
17	649–657	Thiazins	3	0	1	4	5
18	658–663	Thiobenzenyl	1	0	0	1	5
19	664–667	Quinolin	1	0	0	1	3
20	668–688	Sulphids	2	0	0	2	19
21	689–695	Indigos	0	1	1	2	5
		Total	33	32	41	106	589

CONCLUSIONS.

Applying the Schacherl rule, "Other groups which contain harmful or merely suspicious colors must be absolutely excluded," to this table and assuming that all entries in columns 1 and 3 shall be regarded as rendering such colors as "harmful or merely suspicious," it will be found that only one group, namely the Stilbene group, would be permitted under that rule; further, that this rule would admit 17 colors, not one of which has been reported on in the literature as to its physiological action. This state of affairs tends to emphasize the difficulties in the way of any generalization which will be safe so far as public health is concerned and fair to those who use food colors for admittedly legitimate purposes and to make the following recommendation appears to be the only satisfactory way of solving the food-color problem:

> Although it would be possible to draw quite reliable conclusions as to the advisability of employing certain colors for food products on the basis of their chemical constituency, the mode of their manufacture and of the ingredients used in same, nevertheless, I think that by far the safest way would be on the one side to force the dealers of colors intended for food products to sell only such colors with which exhaustive and careful physiological tests have been made by experienced and especially impartial and thoroughly reliable people, thereby establishing their harmlessness beyond a doubt. On the other hand, the manufacturers and canners of food products of any description should be forced to purchase and use only those colors which they are sure have been submitted to such careful tests as have been described and by these tests found to be harmless. (*Lieber, The use of coal-tar colors in food products, 1904, p. 150.*)

This view is confirmed by Santori[1] (*Moleschott's Untersuchungen, 1895, Vol. XV, p. 57*), who says:

> From all these experiments it follows that it is impossible, as some have desired to do, to conclude simply from the chemical composition and constitution whether any given coal-tar dye is poisonous or nonpoisonous. Thus Indulin belongs to the same group as Printing Blue and Methyl Violet to the same group as does Acid Violet; therefore each individual coal-tar dye must be separately examined, and it is only by this laborious method that the use of all really poisonous coal-tar dyes will be prevented.

VII. RECOMMENDATIONS MADE BY UNITED STATES COLOR INDUSTRIES AND TRADES TO THE DEPARTMENT OF AGRICULTURE.

Prior to the issuance of any regulations, the commission on rules and regulations for the food and drugs act, June 30, 1906, held meetings in New York City during September of that year. The stenographic reports of those meetings, and the briefs filed, in so far as they relate to colored food, colored food products, or material for coloring foods or food products, have been condensed verbatim in the following pages with the sole exception of omissions, as indicated; for obvious reasons the names of those making the suggestions hereinafter quoted are not given. This review of opinions expressed and recommendations given by the industries and trades most interested in the manufacture, use, and sale of food colors and colored food products is believed to be fair and full with respect to each and every quotation. The numbers in parentheses following each quotation refer to the pages in the stenographic minutes of the hearing from which those quotations are made.

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[1] Santori examined 15 different blue and violet dyes on dogs by the mouth and hypodermically. Of these 15, 8 are poisonous by the mouth and 7 are nonpoisonous by the mouth. He found Indulin to be poisonous and Printing Blue to be nonpoisonous; Acid Violet to be nonpoisonous and Methyl Violet to be poisonous.

ANTAGONISTIC TO ALL ADDED ARTIFICIAL COLOR.

1. Our position in the coloring question is that we are opposed to all artificial coloring matter in food products. * * * (p. 109).

2. Secondly, speaking first of our own business, and I believe that my view would hold as regards all food products, it is my opinion that all added artificial coloring matter in food products should be prohibited. My experience in our line of business demonstrates this to me beyond any question of doubt (p. 439).

CONCERNING RESTRICTIONS AND REQUIREMENTS.

WHAT CLASSES OF COLORS SHOULD BE PERMITTED TO BE USED.

1. All colors, irrespective of their class, whether animal, vegetable, or synthetic, which have been physiologically and chemically examined, and which will neither retard digestion nor have special physiological effects when consumed in quantities corresponding to two grains per day per adult (p. 106).

2. On the use of colors we recommend that any kind of a harmless color should be permitted provided it is not a color generally known to be poisonous, or generally found to be poisonous, or one that may be almost impossible to be produced without containing some poison within itself, when finished and ready for use (p. 119).

3. Only such colors as are guaranteed to be harmless by reliable manufacturers should be used in the manufacture of confectionery (p. 555).

WHY COAL-TAR COLORS SHOULD NOT BE BARRED.

4. Coal-tar colors, as a class, should not be prohibited; but all those coal-tar colors generally found to be poisonous, or which are hard to produce without containing poisonous properties when ready for use, should be forbidden the privilege of being used or offered for sale for use in food (pp. 116, 117).

5. I ought to put in a plea for the use of coal-tar colors, harmless, of course, for the reason that we have not as yet been able to find any vegetable coloring that is suitable that will give us the results that we require. Coal-tar colors, as everybody knows, are much stronger and are more soluble and not acted upon by acids, whereas the vegetable colors, with but one exception, which is a dark red, we have found great difficulty in making use of for bottled soda water. Almost all the vegetable colors either fade out or change on account of the citric acid in the syrup or food, or form in a little while a precipitation which renders the goods unsalable (pp. 119, 120).

6. * * * aniline butter colorings * * * are superior to all vegetable colors in the following points: (1) Shade and brilliancy. (2) Strength, by which less foreign material is introduced into the butter. (3) Permanency when exposed to light and cold storage. (4) No effect on the taste or flavor. (5) A clear solution

without sediment or mud which gives the butter a uniform tint without specks (pp. 176, 177).

7. We recommend * * * that the use of harmless coal-tar colors, such as chrysoidine, tropæoline, azoflavine, rocelline, ponceau, Bordeaux, Biebrich red, sulphonated fuchsin and naphthol yellow S be allowed, subject to declaration on the label of the quality and quantity of the color used (p. 226).

RELATION OF LABEL TO COLORED FOOD PRODUCTS.

8. * * * if it seems wise in the opinion of the commission to allow certain coloring matters in food products, then the names of the coloring matter ought to be stated on the article (p. 109).

9. * * * I suggest that the use of aniline colors should be made proper if it is so stated on the label (p. 127).

10. We recommend * * * that the use of harmless coal-tar colors * * * be allowed subject to declaration on the label of the quality and quantity of the color used (p. 226).

LABELING OF FOOD COLORS AS DISTINGUISHED FROM COLORS FOR OTHER USES.

11. We recommend that if by any means the Government has the power under the food laws to compel color manufacturers so to do, they be compelled to label all packages containing colors intended to be used in articles of food as colors intended for such purposes as distinguished from colors intended for other purposes; * * * (pp. 555, 555*a*).

THE TEST OF A HARMLESS COLOR.

12. A harmless color is one "which will neither retard digestion nor have special physiological effects when consumed in quantities corresponding to two grains per day per adult" (p. 106).

QUANTITY OF COLOR TO BE CONSIDERED IN DETERMINING HARMLESSNESS.

13. The quantity of 2 grains is mentioned here because in confectionery where these harmless colors are more used perhaps than in any other product, it would be a proportion of one part of color to 3,500 parts, representing 1 pound of color to 3,500 pounds of confectionery, and that is why that was accepted, because that is practically the maximum quantity used in confectionery (p. 106).

14. In the very minute quantities in which the colors are used in carbonated beverages, it would seem a great hardship to prevent us from using coal-tar colors. One ounce of coal-tar red will color satisfactorily from 1,000 to 2,000 gallons of soda water. Of yellow and orange 1 ounce will color from 1,000 to 8,000 gallons. It is readily seen that unless the coloring used is absolutely a violent poison it can have absolutely no effect on the consumer who takes it in an 8-ounce glass, and who could not possibly consume half a gallon or a gallon of that product (p. 119).

15. On this basis 1,000 pounds of butter would contain 420 grains of aniline color (p. 183).

16. One ounce of color to 30 pounds colored food (p. 135).

THE NUMBER OF DIFFERENT COAL-TAR COLORS REQUIRED.

17. And if I were on this committee I would advocate taking out two or three dyestuffs which beyond any shadow of doubt are harmless, and which have been experimented upon, and which would be sufficient for all the purposes of the industry * * * none of these colors are patented, anybody can manufacture them (pp. 147, 148).

18. You have been told that the food commission of the State of Pennsylvania is going to rule that seven colors shall be allowed in the State of Pennsylvania. That is not enough, because it is not possible to reproduce all of the required shades with those colors unless you take the seven primary colors, when you can reproduce any colors. But it is absolutely necessary to have about three yellows to meet the requirements of the trade. Some of the articles that are put out have an organic acid nature, and the anilines will stand that. * * * On that account the character of the food product must be taken into account in the use of the color, and the confectioners have about 30 colors that I know positively of * * * (p. 160).

19. We recommend that * * * chrysoidine, tropaeoline, azoflavine, rocelline, ponceau, Bordeaux, Biebrich red, sulphonated fuchsin, naphthol yellow S be allowed * * * (p. 226).

MANUFACTURING REQUIREMENTS WHICH COAL-TAR COLORS MUST FULFIL.

20. Coal-tar colors, as everybody knows, are much stronger and are more soluble and are not acted on by acids. * * * Almost all the vegetable colors either fade out or change on account of the citric acid in the syrup or food, or form in a little while a precipitation which renders the goods unsalable (p. 119).

21. There are many of these colors (coal-tar colors) that will not stand the natural acids produced in manufacturing foods. For instance, in making confectionery, in boiling candy you make a certain grade of candy where the mixture is boiled to 230°. A certain color will stand that temperature. Then you take another candy and that is boiled to 320° or 340° F., and the colors that will stand 230° will in many cases not stand the temperature of 340°, while another class of colors will stand that temperature. So you have got to distinguish and get a color that will stand these difficult requirements (pp. 159, 160).

GUARANTEES AND GOVERNMENT CONTROL.

22. Only such colors as are guaranteed to be harmless by reliable manufacturers should be used in the manufacture of confectionery (p. 555).

23. We recommend * * * that the Government procure samples of such colors from time to time, wherever they have jurisdiction so to do, and if such colors be not legal for such purposes under the food law, that the same be prosecuted and driven off the market in so far as the Government has the power to do so; and if no means can be devised to compel such labeling of colors intended for food purposes, then that the Government procure such evidence as possible as to the purpose for which a color is intended to be used, and if such evidence shows a food purpose, that such color be prosecuted if illegal under the food law (pp. 555, 555 a).

24. See page 13.

25. The chemical test is the first, and that might throw out a color on account of its containing a little tin or zinc, or some substance foreign to the food product. Whether that is deleterious in the quantity in which it is present or not is immaterial (p. 160).

26. In a brief filed the following suggestions were made:

It must be stated that all the chemist can determine is whether or not the colors contain some impurities that are known to be of poisonous nature. Aniline colors are or can be made entirely free of such impurities, and with this fact established the task with the chemist is exhausted. When it comes to decide the question whether or not a color by itself, when free of all impurities, is injurious to health or not, then the chemist is not the proper authority; it is for the physiologist and for the medical profession to pass on such questions. Chemical theories go for nothing in deciding such questions. It would not even do to classify colors or other substances according to their makeup, as it has been shown again and again that substances belonging to the same chemical class are entirely different in regard to the physiological conduct, Reliable information on this subject can be gained only by physiological experiment, as we can not say definitely whether a substance, color or any other, is injurious or not without finding out for every substance by experiment. This has been done for a considerable number of aniline colors, and these experiments are the only things that deserve any attention. Everything else is idle talk. * * * There are a very large number of aniline colors that have not been treated yet, and we are safe in saying that among these will be also some harmless and others injurious. As they have not been experimentally tested, we do not know which are harmless and which are not; It will therefore be clear that a law forbidding the indiscriminate use of aniline color for the purpose of coloring articles of food is necessary and useful.

But if the meaning of the law is to prevent only the use of injurious colors, as it appears to be, then the way to proceed would be very definitely outlined. Besides physiological colors, all such colors should be forbidden that have been found to be injurious and such aniline colors as have not been tested sufficiently. There will then be left over a number of aniline colors which have been proven by experiment to be entirely harmless, even if taken in large doses.

The experimentors were quite impartial. They had no preconceived ideas, but started simply to find out the true state of affairs. Their reports are therefore very reliable, and it will not do to overlook or to ignore them. The colors that they found harmless can be considered perfectly safe, so much the more as the doses conveniently taken with food would be much smaller than the doses that have proven to be harmless. These harmless aniline colors carry all the shades wanted in the food industry. The law should provide that one of these colors (or mixtures of these) must be used when a food article is being colored, because these few aniline colors are the only colors that can be considered perfectly safe as far as our present knowledge goes. Nothing should be left to guesswork or experimenting, as is the case just now. The colors that are permitted should be enumerated by their scientific as well as by their commercial names, and only such colors should be listed as permissible for coloring food products as have been proven to be harmless, even in large doses. Provision should be made to insure the purity of the colors sold for coloring food; the manner of packing such colors and the labeling of same should be laid down clearly, and all colors now listed, aniline as well as physiological colors, should be strictly forbidden. If the problem is viewed without preoccupation and prejudice, the facts given above will speak for themselves.

VIII. INVESTIGATIONS, OTHER THAN ON ANIMALS, BEARING ON THE HARMFULNESS OF COAL-TAR COLORS.

PFEFFER.

Pfeffer, writing on the Absorption of Anilin Colors by Living Cells, summarizes his results as follows:

The relatively little poisonous Methylene Blue does damage protoplasm in a solution of 0.001 per cent.

Methyl Violet: This coloring matter is not only stored up in the juices of the cell, but is also able to color the living protoplasm, and care is necessary, on account of the poisonous nature of the Methyl Violet, to prevent damage; these cautions are based upon solutions of 0.0003 to 0.00001 per cent strength.

Methyl Violet less poisonous than Cyanin.
Bismarck Brown about as poisonous as Methylene Blue.
Fuchsin as poisonous as Methylene Blue.
Safranin as poisonous as Methylene Blue.
Methyl Orange is poisonous only to a slight degree.
Tropæolin 000, Tropæolin 00, and Rosolic Acid are not poisonous.

Methylene Green as poisonous as Methyl Violet.

Nigrosin as poisonous as Methyl Violet.

Eosin (Tetraiodo fluorescein) kills in 0.1 per cent solution, but lets live 24 hours in 0.01 per cent solution. (*Untersuchungen aus dem Botanischen Institut zu Tuebingen, 1886–88, vol. 2, pp. 179–331.*)

WINOGRADOW.

Winogradow reports on the influence of certain coal-tar colors on digestion, which experiments were carried out in glass. The conclusions arrived at are as follows:[1]

The twelve colors, Safranin (584), Azo Fuchsin G. (93), Coerulein S. (527), Jodeosin (516), Magdala Red (614), Benzopurpurin (277, 278, 279, 307), Ponceau 2R (55), Orange II (86), Phloxin RBN (?), Chrysanilin (532), Azoflavin (92), and Cerise (mixture of 448 and 532), even in amounts of a few milligrams, which in relation to the digestive fluid make up only a few tenths or hundredths of a per cent, exercise a strongly retarding, almost completely inhibitive, action upon the peptic digestion of albumen.

The thirteen colors, Chinolin Yellow (667), Acid Green (434, 435), Azo Acid Yellow (92), Naphthol Yellow (4), Primulin (659), Anilin Orange (87), Metanil Yellow (95), Methylene Green (651), Iodin Green (459), Yellow T (84), Anilin Green (?) Auramin O (425), and Martius Yellow (3), retard the digestive action noticeably, although to a slighter degree than the first 12 colors; in any event they are not indifferent. (*Zts. Nahr. Genussm., 1903, v. 6, p. 589.*)

HEIDENHAIN.

Heidenhain, in his book entitled "Ueber chemische Umsetzungen zwischen Eiweisskorpern und Anilinfarben" (Bonn, Germany, 1902), reports on the behavior of 70 different coal-tar colors, 3 intermediate products, and 4 raw materials toward various albuminoids such as serumalbumen, albumen, and casein.

Of these 70 colors, 21 have been investigated physiologically, and the results embodied in this report; and of these 21, 17 were on the United States market as food colors and 4 were not on this market.

Limiting the attention to the 17 that were offered, 4 of them are among the 7 permitted colors of Food Inspection Decision 76.

In the cases where albumen and casein were used, they were employed in 0.5, 0.1, 0.02, 0.01, and 0.005 per cent solutions, acidified with acetic acid; the coloring matter was employed in a 1 per cent solution in the first strength, in 0.1 per cent solution in the second and third strengths, and in a 0.02 per cent solution in the fourth and fifth strengths, and one volume of coloring-matter solution was brought in contact with five volumes of albuminoid solution.

The following colors precipitated the albuminoid in all the strengths. The numbers in parentheses indicate the Green Table numbers;

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[1] It is not always possible to identify the trade names given by Winogradow with specific numbers in the Green Tables; the numbers in parentheses after the name indicate the number in the Green Tables wherever that identification could be made with any reasonable certainty; wherever two or more numbers appear, the context indicates that the name might apply to any one or all of them.

the numbers in italics are those of the permitted list of Food Inspection Decision 76: (55) Ponceau 2 R, (65) Fast Red B, (434) Light Green SF bluish, (*56*) Ponceau 3 R, and (106) New Coccin.

The following precipitated in all but the fifth strength: (*107*) Amaranth.

The following precipitated only the first three strengths: (14) Orange G and (*85*) Orange I.

The following precipitated only the first two strengths: (462) Acid Magenta.

For the basic colors the method of testing was different from that described for the acid colors and the amounts employed were not so definitely set forth. A 1 per cent solution of serum albuminoid was employed; the solution of coloring matter used varied in strength from 0.5 to 1 per cent (*b*), a "very dilute solution" (*a*) being also employed. The annotations given by the author (p. 114) are herewith reproduced in full:

(17) Chrysoidin Y. (*a*) In a very dilute solution the yellow base is at once liberated, and when sufficient color is added albumen precipitation takes place. No color change on heating. (*b*) Turns yellow at once. Further additional color produces a brown and albumen precipitation.

(201) Manchester Brown. (*a*) Becomes yellow at once, due to separation of the free base. On heating no change. (*b*) Becomes a discolored brown, and produces a nice brown albumen precipitate.

(425) Auramin O. (*a*) No change. (*b*) Precipitates albumen strongly.

(427) Malachite Green. (The oxalate of the color was used.) (*a*) Color changes from a blue-green to a more pure green. (*b*) Cold, no precipitation of albumen; heated, sudden precipitation of albumen.

(428) Brilliant Green. (A sulphate of the color was used.) (*a*) Becomes milky. (*b*) Immediate precipitation of albumen.

(448) Magenta. (Acetate and nitrate were used, and in both acted the same.) (*a*) Color changes from yellowish-red to rose-red. (*b*) Albumen precipitation abundant, even in the cold.

(451) Crystal Violet. (*a*) The color loses its reddish cast and changes to pure blue. (*b*) Albumen precipitated by the use of much color.

(*4*) Naphthol Yellow S was tested as the free-color acid and not as the sodium or potassium salt, which is its commercial form. It precipitated the albuminoid from the following solutions: 1. 1 per cent serum albumen in water. 2. 0.5 per cent serum albumen in 10 per cent acetic acid. 3. Casein, 0.5 per cent in 10 per cent acetic acid. 4, Serum albumen; 5, Casein; 6, Nuclein, all in 0.5 per cent solution in 0.2 per cent sodium hydroxid. 7. Nucleinic acid in 0.5 per cent water solution.

The relationship between the amounts of color-acid and albuminoid solution here used does not appear definitely in the book.

Having reference now to the literature and the physiological action, as compiled herein, it will be observed that the ability to precipitate albumen, or not to precipitate it, under the conditions of Heidenhain, appears not to have any direct connection with the results obtained by actual physiological test on animals or man. For instance, among the five that precipitated all five strengths of albumen and casein, No. 65, Fast Red B, of the Green Tables, has been found to be not harmful by tests actually described; No. 56, Ponceau 3 R, belongs to a class of colors generically permitted by the law of Austria; Nos. 55, Ponceau 2 R, and 106, New Coccine, have been reported on both favorably and unfavorably by different experimenters; No. 434, Light Green SF bluish on physiological examination has been described as suspicious.

From this it appears that two colors, physiologically probably harmless, precipitated all the five strengths of albumen, and three colors, which are perhaps no more than suspicious, likewise precipitated all five strengths of albumen.

No. *107*, Amaranth, which has been examined with favorable results by two different experimenters, precipitates four out of the five strengths.

Of the two colors precipitating the first three strengths, both have been examined physiologically with favorable results, namely, Nos. 14, Orange G, and *85*, Orange I.

The color which precipitated only the first two strengths, namely, No. 462, Acid Magenta, has likewise been examined physiologically, and the reports are favorable.

Thus it would seem that there is no definite connection between the physiological action and the ability to precipitate albumen from acetic acid solution in the case of acid colors.

In the case of basic colors the situation seems to be somewhat different.

OTHER AUTHORS.

Rosenstiehl (*Fifth International Congress of Applied Chemistry, Vol. III, p. 700*) states that when the color is present in an excess, yeast absorbs 8 per cent of Magenta (448) and 5 per cent of Malachite Green (427, 428). The Acridins (528–538), the Thionins (649–657), the Safranins (583, 584), and the Rosanilins (447–448) dye yeast the best; solutions containing 3 per cent by weight of the dry weight of the yeast are completely decolorized by such yeast and at ordinary temperatures. The Eosins and Phthaleins dye the yeast only incompletely, whereas Azo dyes (7–393) (with the exception of Benzo Purpurin, 277, 278, 279, 309) do not dye yeast at all. Such dyed yeast, however, is not dead; it has merely lost its power to cause fermentation. The numbers in parentheses are the corresponding numbers in the Green Tables as nearly as they could be identified.

Bokorny (*Chem. Ztg., 1906, v. 30, p. 217*) examined Magenta (448), Safranin (584), Victoria Blue (487, 488 or 490), Methylene Blue (650), and Alizarin Blue (562 or 563) (the numbers in parentheses are the probable Green Table numbers) as to their effect on micro-organisms such as yeast cells, infusoria, and the like, and found that these dyes in a concentration of 1:100,000 killed them, whereas strychnin nitrate in the same concentration is substantially without effect. Death is caused by absorption of the dye by the albumen of the protoplasm. The dyes seem to be absorbed not only by living albumen, but also by living nerve cells and fibers. Pure anilin or coal-tar colors, however, are not poisonous in the ordinary meaning of the words, that is, humans are not likely to be easily injured by them.

Houghton (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–1357*) shows that Bismarck Brown (197; 201) and Crocein Scarlet (160?) hinder the peptic digestion of fibrin, casein, and albumen.

Stilling (*Anilinfarbstoffe als Antiseptica, 1890, v. 2, pp. 55–56*) states that he found the animal cells to be affected by pure coal-tar colors in the same way that vegetable cells were affected.

Penzoldt, based upon the experimental work of Beckh (*Archiv. Exp. Path. Pharmak. 1890, v. 26, p. 310*), reports as follows:

Of the 15 dyes—

	Name.	Green Table number.
1.	Malachite Green	427
2.	Hofman's Violet (methyl variety)	450
3.	Methyl Violet	451
4.	Rose Bengal (Erythrosin)	*517*
5.	Phenyl Blue	480
6.	Methylene Blue	650
7.	Fuchsin	448
8.	Coralline	484
9.	Eosin	512
10.	Methyl Orange	87
11.	Vesuvin	197
12.	Tropaeolin	88
13.	Scarlet Red	(?)
14.	Congo Red	240
15.	Indulin sulpho acid	601

all of which are water soluble and when used were free from arsenic, only the first six in saturated solution arrested the development of *Staphylococus pyogenes aureus*, and of these six all but the Erythrosin and Methylene Blue arrested the growth of anthrax bacillus.

Of these six when injected into rabbits subcutaneously the following produced no changes of consequence: Erythrosin (250), Phenyl Blue (100), Methylene Blue (75). The numbers in parentheses show the number of milligrams of dye per kilo body weight of the rabbits.

Methyl Violet (50) produced only local changes, such as gangrene.

Malachite Green (100) and Hofman's Violet (20) produced muscular paralysis, which in the case of Malachite Green resulted fatally on the ninth day; in the case of Hofman's Violet the paralysis was complete on the tenth day.

The remaining nine colors are apparently of no effect upon staphylococus or upon anthrax.

H. W. Williams (*A Manual of Bacteriology, 1906, p. 200*), under "Disinfectants and Germicides," says:

> Aniline dyes. Many of these substances, notably pyoktanin (Methyl Violet), possess germicidal properties. A solution of 1:5000 will kill the anthrax bacillus in two

hours. A much stronger solution, 1:150, is required to kill the typhoid bacillus in the same time. Malachite Green is said to possess even greater germicidal power than pyoktanin. Methylene Blue also possesses considerable germicidal power.

IX. COMPILATION UNDER THE GREEN TABLE NUMBERS OF ALL INFORMATION AVAILABLE AS TO THE SUITABILITY OF COAL-TAR COLORS FOR FOOD.

GENERAL STATEMENTS.

Before entering upon a detailed study of what has been published for and against specific coal-tar colors, it is probably well to consider, for whatever they may be worth, some of the general statements that have been made, from time to time, in the literature relative to coal-tar colors, considered either as a whole or as subdivisions or classes thereof, and their physiological action or their suitability for use in foods.

1. Schultz (*Die Chemie des Steinkohlentheers, Brunswick, 1887–1890, Vol. II, p. 35*), after discussing the regulations of the German Empire in respect to food coloring, says:

> With respect to these regulations the artificial organic coloring matters can be regarded, in general, as harmless. For the purposes of dyeing magenta made by means of arsenic acid, further picric acid, and those coloring matters which occur as oxalates or zinc chlorid double salts, such as Methylene Blue and Bitter Almond Oil Green, can be used. The use of the substances named for the coloring of food products is, however, suspicious and should not be permitted.

2. Stilling (*Anilinfarbstoffe als Antiseptica, Strassburg, 1890, Vol. II, pp. 55–56*) says:

> In view of the fact that the most innocent substance, such as distilled water or common salt, when introduced in large quantities into the organism can act fatally, the anilin coloring matters therefore, particularly if they be free from all admixtures, such as arsenic, copper, and chlorid of zinc, are to be regarded as wholly nonpoisonous.
>
> All experience gathered since my first publication has likewise fully confirmed this nonpoisonous nature.

3. Stilling (*Ber. Klin. Wochensch. 1890, p. 531*) also says:

> Proceeding from purely theoretical views, and based upon these botanical and physiological experiments, I have recommended anilin colors as antiseptics for the following reasons:
>
> 1. They are to be designated as absolutely nonpoisonous. This will be confirmed by every chemist acquainted with the relevant details, and also from the medical side this has long ago been determined by Grandhomme. The publications of this author, who made extensive observations and experiments in the anilin factory of Meister, Lucius & Bruening, appeared in the beginning of the eighties, and has hardly become known in medical circles. However, I was first made acquainted with this by my colleague, Prof. Rose, in Strasburg.
>
> 2. That it is possible to bring about death in experimental animals by introducing large amounts of the coloring matter into the peritoneal cavity proves nothing against

the nonpoisonous nature of these substances. This action is to be regarded as a purely mechanical one, a view which I will thoroughly confirm in my second communication.

With respect to the anilin colors not soluble in water, Ehrlich long before me, in his excellent publication on the oxygen requirements of the organism, has arrived at this view, and has excellently described it as merely a penetration of the organs.

4. Lehmann (*Methoden der Praktischen Hygiene, Wiesbaden, 1890, p. 543*) says:

> The hygienic significance of coal-tar colors has heretofore been judged quite variously. When the intensely poisonous nature of the first impure and particularly arsenic-containing coloring matters became known the inclination was to judge the coal-tar coloring matters very strictly; when it was subsequently recognized that the contaminations were largely the cause of the harmfulness to health, there followed a period in which no poisonous coal-tar coloring matter was known in any pure condition. (Eulenberg & Vohl, 1870.) More recent investigations, however, have disclosed a series of coal-tar coloring matters which, as a matter of fact, possess a considerable poisonous action, and already cases, although not numerous, have become known in which serious and even fatal poisonings by means of pure coal-tar colors have arisen. Alongside of this there still continue to exist the possibilities described by Eulenberg and Vohl (Viertel-Jahressch. für Gerichtliche Mediz, 1870), whereby harmless coloring matters become harmful; but the realization of these possibilities has become essentially more seldom through improvements and changes in manufacture.

5. Stilling (*Arch. Exper. Pathol. Pharmak., 1891, v. 28, p. 352*), in speaking of the anilin colors as antiseptics, says:

> It is the nonpoisonous nature of these substances, their easy solubility and diffusibility, and above all their inability to coagulate albumen which lends them their importance, which now can be only difficultly denied.

NOTE.—The work of Heidenhain abstracted in Section VIII does not fully bear out this article.

6. Erdmann (*Pharm. Centralh., 1892, v. 33, p. 357*) says:

> The sulphonated as well as the carboxylated coal-tar dyes will not have any pronounced action on the organism. Acid dyes may therefore be regarded in general as nonpoisonous, whereas in the case of basic dyes a physiological examination is to be recommended before they are permitted to be applied to articles in daily use or indeed to be used in food or drink.

NOTE.—Out of the 80 different dyes on the food-color market in the summer of 1907 whose composition was avowed, 15 were basic and 65 were acid.

7. Tschirch expressed himself as follows:

> 1. The coal-tar colors, and in a narrower sense the anilin colors, are no longer harmful on account of their arsenic content, since at the present time the great majority of them are prepared free from arsenic.
>
> 2. Some colors have shown themselves to be harmful to the system.
>
> 3. Coal-tar colors, in general, should therefore be permitted for the coloring of foods, but those that have been found to be harmful should be expressly and specifically forbidden.

4. The amount of coloring matter which has been determined quantitatively in bonbons and liqueurs is so small that even the ones regarded as poisonous would not be able to develop their harmful effects. (*Zts. Nahrs. Unters. Hygiene Waarenk., 1893, v. 7, p. 338.*)

8. Georgievics (*Lehrbuch der Farbenchemie, Leipzig, 1895 p. 10*) says under "Poisonous nature of the coloring matters:"

It is a little known fact that of the very large number of organic coloring matters only a few have been found to be poisonous; these are Picric Acid, Victoria Orange (Saffron-surrogate), Aurantia, Metanil Yellow, Orange II, and Safranin. The prejudice which is still quite widely accepted that most of the artificial coloring matters are poisonous dates from the early periods of anilin-color manufacture, at which time magenta and the coloring matters made from it occurred in commerce highly contaminated with arsenic. At present, however, these coloring matters are prepared absolutely free from arsenic, and are, as such, nonpoisonous. A few coloring matters which occur commercially as zinc chlorid double salts, such as Methylene Blue and Malachite Green, may be harmful in consequence of their zinc content, and should therefore never be employed in the coloring of food products. * * * In consequence of their physiological activity some coloring matters are employed as medicines, indeed principally Methyl Violet, Auramin and Methylene Blue. The first two, known as blue and yellow Pyoctanin (pus-destroying) are, owing to their great antiseptic action and diffusibility, valuable medicines; on account of the unpleasant coloring effect accompanying them they are but little used. Methyl Violet was first recommended as an antibacterial remedy in diseases of the eye by Stilling; subsequently it has been employed in other special cases; its principal use, however, is in surgery for the prevention of malignant proud flesh.

The use of Auramin is entirely analogous.

Methylene Blue (as a free base) is used principally as an analgesic (pain-relieving remedy) and is given internally; on account of its ability rapidly to diffuse through the tissues of the nervous system; it can also be introduced by injection. It is used as a remedy against malaria, carcinoma, Bright's disease, etc.

The following have been tested as remedies or as antiseptics: Safranin, Lydin (Mauvein), Vesuvin, Anilin Blue, Carbolic Magenta, Alizarin Yellow C (Gallacetophenone), etc. The potassium salt of Dinitro-ortho-cresol was brought into commerce by the Farbenfabriken vorm. Friedr. Bayer & Co., under the name of Antinonnin, and has given excellent results as a means against formation of mold in cellars and against wood fungi.

9. Weyl (*Handbuch der Hygiene, 1896, p. 378*) says:

A few organic coloring matters, but only a very few, possess poisonous properties. A rule by means of which the poisonous or nonpoisonous nature of organic coloring materials can be determined without experiment is unknown even for those coloring matters whose constitution has been determined and experiments on the poisonous nature of organic coloring matters are very few in number.

10. Lewin (*Lehrbuch der Toxicologie, 1897, p. 230*) says:

In the use of various fabrics or of foodstuffs, which are colored with anilin or coal-tar colors, or in commercial contact with such colors, local and general symptoms of poisoning, such as eczema, swelling of the face, vomiting, diarrhea, anæsthesia, paresis, etc., have been observed. These are generally due to the toxic nature of the coloring matters, frequently to harmful ingredients of the same, and hardly ever to poisonous mordants. Many workmen in anilin factories show permanent spots; for example, on the cornea and conjunctiva, head, chest, face, and neck, without any interference with their general condition. Local changes of more serious nature have more frequently been observed in the mucous membranes and on the skin. Thus, in one case, a camel's-hair pencil, soaked with anilin color, accidently entered the eye, and at first nothing was noticed but a violet blue coloration, later inflammaion and chemosis took place. I have observed local swelling and indurations of the skin, particularly on the cheeks, in the case of children after they had worn caps colored with anilin dye.

11. Winton (*Connecticut Agric. Exper. Sta. Report, 1901, p. 181*) says:

Although there is evidence that most of the coal-tar dyes are not injurious to some of the lower animals, it is not safe to assume that they are entirely harmless to human beings. The dog, an animal used in most of Weyl's experiments, has a proverbially strong stomach, and eats with no apparent discomfort many things which would disturb the digestion of a man.

12. Chlopin, in his book published in 1903 (see p. 75), states as follows:

(*Page 114.*) * * * All the dyes examined by me I divided into three categories: Dyes which caused striking general symptoms of poisoning and led to the death of the animal, or would have led to it if the experiments were not purposely discontinued, I designate by the term poisonous; dyes which induced some separate and temporary symptoms of disease, for instance, vomiting, diarrhea, separation of albumen in the urine, the general condition remaining normal, I designate as suspicious; lastly, the dyes which caused no apparent disturbance during the experiments are designated by the term nonpoisonous. I purposely do not call the last category harmless, because by our experiments the question could not be decided negatively as to whether the nonpoisonous dyes did not cause some finer pathological changes in the organism and functions which could not be detected by simple observation.

(*Pages 219–221.*) Thus, according to all the investigators quoted, there were found altogether 22 poisonous and harmful dyes, out of about 60 dyes examined; which makes 36.7 per cent of poisonous and harmful dyes among those examined.

My investigations gave 30 per cent of poisonous and 40 per cent of suspicious dyes.

The percentages above given have a fairly well established basis, since they were obtained by the examination of 100 dyes, which is about one-fifth of all the dyes in commerce. Further, considering the distribution of the poisonous and harmful dyes according to various chemical groups, we find that they occur in 12 of the 18 groups, and we can not note any regularity in this distribution; it is impossible to say that there is any definite connection between the fact that the dye belongs to a certain chemical group and its action on the animal organism. Usually among the dyes of one and the same group there are some harmful ones, but there are also some harmless ones, and the ones and the others have very similar composition. This or that action of the dyes on the animal organism, as we shall presently see, is determined more by the delicate difference in the internal structure of their molecules than by those differences on which is based, at the present time, the classification of the aromatic dyes.

On the basis of the whole experimental material on hand—mine and that of other investigators—I can make only the following very few and purely empirical generalizations:

1. According to the shade produced, the poisonous and harmful colors are distributed as follows: Most of all poisonous dyes are found among the Yellows and the Oranges; then come the Blues, then the Browns and the Blacks; there are very few harmful dyes among the Violets and Greens; among the Reds was found only one suspicious one, and no poisonous ones.

2. The most poisonous dyes belong to the Nitro, Azo, Triphenyl, and Thiazin groups, and also to the Auramins.

3. A whole group of poisonous and harmful dyes is formed by the new sulphid dyes known as Vidal's dyes.

(*Page 224.*) By the facts and observations quoted above is corroborated the opinion that in general the coal-tar dyes, according to the composition and properties, appear as substances foreign to the animal organism, and may influence harmfully the vital functions, even in those cases when they do not possess distinctly poisonous properties. For this reason many hygienists make it a principle not to allow the coloring of food products or of beverages with coal-tar dyes, independently of the fact whether they prove in actual experiments on animals poisonous or not.

We must therefore agree with M. Rudner that food of the masses require the most far-reaching protection, maintaining them free from foreign additions.

13. Koenig (*Die Menschlichen Nahrungs- und Genussmittel, Berlin, 1904, Vol. II, p. 462*) says:

Even though the majority of the anilin coloring matters, in view of the small amounts in which they are generally employed, can not be regarded as directly harmful to health, yet the objections to their use in the coloring of food products for the purpose of substituting or strengthening a natural color lies in the deception connected therewith * * *.

14. Fraenkel (*Arzneimittel-Synthese, Berlin, 1906, p. 570*) says:

It is clear that the coloring property of these chemical substances stands in no relation to their physiological actions, but, on the contrary, the physiological actions depend only upon the general structure of these substances, and therefore upon their membership in definite chemical groups.

(*Pages 574–5.*) We see, even in considering this group of substances, that they do not possess any specific action, but they are capable of use, preferably by external application, as antiseptic materials, as materials which in their action stand somewhere between carbolic acid and corrosive sublimate, and whose coloring property, in consequence of which they were primarily selected, is directly a hindrance in this use, since the coloring of the bandages and the hands of the operators and the skin of the patients certainly can not be regarded as a pleasant occurrence; that the antiseptic action stands in some relationship to the properties of the substances as coloring matter must be positively contradicted. It depends only on the general structure of the substance, and does not stand in any direct relation to the chromophore and auxochrome groups of the substances, but more closely to the aromatic nucleus. Indeed, it may happen that an auxochrome group diminishes the antiseptic activity of such a substance.

NOTE.—The dyes referred to belong to the Monazo, Disazo, Triphenyl-methane, Xanthin, Azin, and Thiazin classes.

(*Page 92.*) The investigations of Ehrlich have shown that basic dyes color the brain gray, and, moreover, they color nerve fiber very well, and are therefore to be regarded as neurotropes. The dye acids, on the other hand, do not dye nerve fiber, and in particular the substituted sulphonic acids do not dye tissue at all.

15. Meyer, in his paper on "A preliminary communication on the toxicity of some aniline dyestuffs" (*J. Amer. Chem. Soc., 1907, v. 29,* p. 892), says:

(*Page 893.*) "A manufacturing confectioner of this city, for whom I made examination of colors used by him, informs me that a yellow color sold as Auramin has such high tinctorial power that 1 ounce will color 2,000 pounds of candy to the highest yellow tint required in his business. It is obvious that the toxicity of such a body would have to be very high to render it harmful in such use." Conclusions of this kind do not take into account the possible detrimental action ensuing on healthy as well as diseased persons from long-continued use of small quantities of foreign substances.

(*Page 909.*) The same author raises objection to feeding experiments on the ground that substances are thereby introduced greatly in excess of the amounts generally found in foods and that the ill effects "are liable to be due to the excess and in long-continued experiments due to a cumulative action of the excess." Surely if excessive amounts have a cumulative action, small amounts may also finally show toxic effects due to retention and accumulation of the poison. To declare a substance entirely innocuous would require evidence as to its nontoxicity both to normal and diseased persons after its long-continued administration in both small and large doses. The most extreme contingencies would have to be provided for. The above objections to feeding experiments are therefore not valid. It is hoped that a study of the effects on metabolism of some of these substances will help to further elucidate the subject.

He summarizes the results of his physiological investigations of seven different coal-tar colors as follows:

> 1. Several commercial organic dyestuffs (Curcumin S, Tartrazin, Naphthol Red S, Carmois in B, Naphthol Yellow S, Gold Orange, and Ponceau 2 R) were studied as to their general effects on dogs when administered in varying amounts and during fairly long periods (two weeks).
> 2. None of these dyestuffs under the conditions above indicated exhibited any marked degree of toxicity. There was only one fatal result, which may have been due to influence independent of the action of the colorant.

Similar quotations from the literature could be added to the foregoing, but these are beyond question sufficient to show that a wide divergence of opinion as to the harmless or harmful nature of the coal-tar colors as a class does exist among scientific men, and that all those above quoted agree that there are some at least of the coal-tar colors which even in a pure state may be harmful to human health, and that the question of actual harmfulness under the conditions of actual use in foods and the consumption of foods is regarded by some as being properly answered in the negative and by others as being properly answered in the affirmative. The question of amount of color employed in the food products and the amounts of such food normally eaten are therefore raised by some as the deciding factors.

In this connection the following statement from page 49 of the arguments before the Committee on Patents in the House of Representatives, April 8, 15, 16, 22, 29, 1908, may be of interest:

> * * * It should be remembered that after a new chemical has been discovered and patented it requires as many as three years of experiment before we dare offer it in this country as a medicine for human beings. These experiments are conducted abroad before we receive it here. It is first tried on animals and gradually, with great caution, extended to human beings in the foreign hospitals, so as to ascertain its physiological effects quantitatively upon the various organs, both when those organs are in the healthy state and when they are affected by various disorders * * *.

A search of the literature herein compiled fails to disclose any such searching physiological examination of any of the coal-tar colors recommended for use by human beings in food products, as is asserted in the above quotation to be necessary in the case of a new chemical intended for use as a drug. If Fraenkel, as quoted on page 60, is correct in his statement that coal-tar colors act physiologically because they are chemicals and not because they are coloring matters, then coal-tar coloring matters prior to use in foods, in which they are used by the young and the old, the well and the sick, without restriction and without supervision, should also be thoroughly tested, and very few, if any, coal-tar colors seem to have been examined with the thoroughness set forth in the above quotation. That uniformity and purity of product is necessary in order to be sure that the chemical is going to act physiologically in the same way every time is obvious. According to Fraenkel, what is true of a chemical is just as true of a coal-tar color, and if uniformity of strength and cleanliness of product are desirable when a chemical is to be used as a medicine, such properties are equally desirable when a chemical is to be used as an ingredient in food.

CLASSIFICATION OF OPINIONS IN LITERATURE AND IN LEGAL ENACTMENTS SHOWING CONDITION OF THE MARKET IN 1907.

The literature and legal enactments hereinafter grouped under the relevant Green Table numbers have been classified as (1) unfavorable—i. e., only unfavorable reports found in the literature; (2) favorable, and (3) contradictory reports, as each case required; so that under each Green Table entry there is not only the relevant literature, but also the character assigned to it for the purpose of coming to a conclusion as to the propriety of the use of such color in foods as based on such literature, which conclusion formed in that respect the basis for Food Inspection Decisions Nos. 76, 77, and 106.

However, it does not follow that all dyes placed in the "favorable" list are actually harmless; the investigations or opinions reported of each may very well be based upon insufficient data. This classification, therefore, is merely intended to reflect the present state of the literature with respect thereto, and is not necessarily final nor conclusive.

In substantially all the recorded cases the observers directly or indirectly assert the absence of arsenic and mineral poisons in the dyes subjected to physiological test, but the kind of other impurity, if any, is not stated.

For the purpose of a comprehensive survey of the literature and legal enactments, the following tabulation is presented:

Condition of the United States color market in the summer of 1907.

[x=not on market. Figures indicate number of dealers offering sample. Cross lines separate the several groups.]

Green Table number.	Unfavorable.	Favorable.	Contradictory.
1	x		
2	x		
3	x		
[1]*4*		10	
5		x	
6	x		
8			5
9			1
11			2
13			6
14			2
15			x
16			x
17			2
18			1
28		x	
41			x
43			x
55			2
65		2	
70			x
78	x		
84			2
85		2	
86			8
87			x
88			x
89		1	
92		x	
93		x	
94	6		
95			2
97	1		
102		x	
103		6	
105		1	
106			5
107		7	
138			x
160			x
163			x
164	x		
166		x	
169		1	
188			1
197			4
201	2		
240		1	
269			1
277	x		
287			1
394		x	
398			2
399		x	
425	3		
427			2
428			3
433		1	
434	1		
435		4	
448			4
450			x
451			5
457			x
459	x		
462		2	
467		x	
477		x	
478	x		
479	x		
480			1
483			x
488, 490	x		
502	2		
504			5
512		3	
516	1		
517		5	
520		2	
521		x	
527		x	
530	x		
532	x		
563			x
572	x		
574	x		
576		x	
584			1
593		x	
599		x	
600		x	
601			1
602	x		
614	x		
620	x		
639	x		
649	x		
650			2
651	x		
654	x		
659	x		
667	1		
670	x		
675	x		
689			x
692		3	
Totals 106 [2]50	33 [2]8	32 [2]16	41 [2]26

[1] Italicized figures are colors in the permitted list, Food Inspection Decision 76.

[2] On United States market in 1907.

This table shows that of the 106 coal-tar dyes examined physiologically only 50 were on the United States market; further, out of 33 "unfavorable" dyes 8, or one-fourth, were on the United States market; out of 32 "favorable" dyes 16, or one-half, were on the United States market, and finally that out of 41 "contradictory" dyes 26, or very nearly two-thirds, were on the United States market in the summer of 1907.

Assuming this classification to be substantially and essentially fair, the only Green Table numbers which are of interest for the present discussion are those classified under "favorable," because any color positively injurious or of doubtful character is considered as being properly excluded from use in food products. The Green numbers classified as "favorable" are 32 in number, as follows: *4;* 5; 28; 65; *85;* 89; 92; 93; 102; 103; 105; *107;* 166; 169; 240; 394; 399; 433; *435;* 462; 467; 477; 512; *517;* 520; 521; 527; 576; 593; 599; 600; *692.*

Of these 32, 16 were on the United States market in the summer of 1907 and their composition disclosed; they are presented in the following table, together with the number of dealers, out of a possible 12, offering them for sale.

Distribution of "favorable" colors on the American market in 1907.

Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.	Green Table number.	Number of dealers handling.
4	10	103	6	240	1	512	3
65	2	105	1	433	1	*517*	5
85	2	*107*	7	*435*	4	520	2
89	1	169	1	462	2	*692*	3

From among these 16, six of the seven permitted colors of Food Inspection Decision No. 76 were selected. The process of selection and of elimination is described on page 166.

CLASSIFICATION ACCORDING TO CHEMICAL COMPOSITION AND SUITABILITY.

In the following table the chemical composition of the substances corresponding to the Green Table numbers is given, and their classification according as the literature is regarded as being (1) unfavorable, (2) favorable, or (3) contradictory in regard to the color. The colors are also arranged in the groups to which they belong chemically, so that this table shows: (1) The number of groups reported on in the literature, (2) the number and composition of members of each group so reported on, and (3) the interpretation here placed upon such reports in literature. This table is given in the expectation that it will be of use to chemists and physiologists.

Opinions as to suitability, classified according to groups and chemical composition.

(The chemical nomenclature is that of the Green Tables; "a" is used for alpha and "b" for beta.)

Unfavorable.	Favorable.	Contradictory.
NITRO COLORS.		
1. Symmetrical trinitrophenol. 2. Dinitro o and p-cresol. 3. Dinitro-a-naphthol. 6. Hexanitro-diphenylamin.	4. Dinitro-a-naphthol-b-monosulphonic acid. 5. Dinitro-a-naphthol-a- monosulphonic acid.	
MONOAZO COLORS.		
78. Methyl-benzenyl-amido-thio-xylenol-azo-a-naphthol disulphonic acid. 94. Benzene-azo-pyrazalone-carboxy-disulphonic acid. 97. Sulpho-o-toluene-azo-b- naphthol.	28. p-nitro-benzene-azo-a-naphthylamin p-sulphonic acid. 65. a-naphthalene-azo-b-naphthol-disulphonic acid. 85. p-sulphobenzene-azo-a-naphthol. 89. p-sulphobenzene-azo-diphenylamin-sulphonic acid. 92. Diphenylamin yellow with nitro-diphenylamin.	8. Amido-azo-benzene-di- and monosulphonic acid. 9. Amido-azo-toluene-disulphonic acid. 11. Benzene-azo-b-naphthol. 13. Benzene-azo-b- naphthol-b-sulphonic acid. 14. Benzene-azo-b-naphthol disulphonic acid G.

Opinions as to suitability, classified according to groups and chemical composition—Con.

Unfavorable.	Favorable.	Contradictory.
MONOAZO COLORS—continued.		
	93. p-sulphobenzene-azo-dioxy-naphthalene sulphonic acid. 102. p-sulpho-naphthalene-azo-b-anphthol. 103. p-sulpho-naphthalene-azo-a-naphthol-p-sulphonic acid. 105. p-sulpho-naphthalene-azo-b-naphthol monosulphonic acid. 107. p-sulpho-naphthalene-azo-b-naphthol-disulphonic acid.	15. Benzene-azo-b-naphthol disulphonic acid R. 16. Dimethyl-amido-azo-benzene. 17. Hydrochlorid of diamido-azo-benzene. 18. Hydrochlorid of benzene-azo-m-tolylene-diamin. 41. Hydrochlorid of toluene-azo-m-tolylene-diamin. 43. Toluene-azo-b-naphthol-sulphonic acid. 55. Xylene-azo-b-naphthol-disulphonic acid. 70. Dichloro-phenol-azo-b-naphthol. 84. p-sulphobenzene-azo-resorcinol. 86. p-sulphobenzene-azo-b-naphthol. 87. p-sulphobenzene-azo-dimethylanilin. 88. p-sulphobenzene-azo-diphenylamin. 95. m-sulphobenzene-azo-diphenylamin. 106. p-sulphonaphthalene-azo-b-naphthol-disulphonic acid.
DISAZO COLORS.		
164. Sulphobenzene-azo-sulphobenzene-azo-b-naphthol-sulphonic acid. 201. Hydrochlorid of toluene dis-azo-m-tolylene-diamin. 277. Ditolyl-disazo-binaphthionic acid.	166. Sulphobenzene-azo-sulphobenzene-azo-p-tolyl-b-naphthylamin. 169. Sulphotoluene-azo-toluene-azo-b-naphthol-a-sulphonic acid. 240. Diphenyl-disazo-binaphthionic acid.	138. Bisulphobenzene-disazo-a-naphthol. 160. Sulphobenzene-azo-benzene-azo-b-naphthol-monosulphonic acid. 163. Sulphobenzene-azo-sulphobenzene-azo-b-naphthol. 188. Disulpho-b-naphthalene-azo-a-naphthalene-azo-b-naphthol-disulphonic acid. 197. Hydrochlorid of benzene-dis-azo-phenylene-diamin. 269. Ditolyl-disazo-bi-salicylic acid. 287. Ditolyl-disazo-bi-a-naphthol-p-sulphonic acid.
NITROSO COLORS.		
	394. Dinitroso-resorcinol.	398. Nitroso-b-naphthol-b-mono-sulphonic acid.
STILBENE COLORS.		
	399. Azoxy-stilbene-di-sulphonic acid.	
DIPHENYLMETHANE COLORS.		
425. Hydrochlorid of imido-tetra-methyl-diamido-diphenyl-methane.		
TRIPHENYLMETHANE COLORS.		
434. Dimethyl-dibenzyl-diamido-triphenyl-carbinol-trisulphonic acid. 459. Chlorid of heptamethyl-rosan-ilin chlorid. 478. Triphenyl-pararosanilin di- and trisulphonic acid. 479. Triphenyl-pararosanilin-tri-sulphonic acid. 488-490. Hydrochlorid of phenyl-tetra- (penta) methyl-triamido-diphenyl-a-naphthyl carbinol.	433. Diethyl-dibenzyl-diamido-triphenyl carbinol-disulphonic acid. 435. Diethyl-dibenzyl-diamido-triphenyl-carbinol-trisulphonic acid. 462. Trisulphonic acid of rosanilin and pararosanilin. 477. Triphenyl-rosanilin-monosulphonic acid and triphenyl-pararosanilin-monosulphonic acid. 487. Disulphonic acid of dimethyl dibenzyl di-ethyl triamido triphenyl carbinol.	427. Chlorid of tetra-methyl di-p-amido-triphenyl-carbinol. 428. Sulphate or chlorid of tetra-ethyl-diamido-triphenyl-carbinol. 448. Hydrochlorid or acetate of pararosanilin and rosanilin. 450. Hydrochlorids or acetates of mono-di or trimethyl (or ethyl) rosanilins and pararosanilins. 451. Hydrochlorid of penta- and hexamethyl-pararosanilin. 457. Hydrochlorid, sulphate or acetate of triphenyl rosanilin and triphenyl pararosanilin. 480. Triphenyl-rosanilin and triphenyl-para-rosanilin sulphonic acids. 483. Aurin carbinol, oxydized aurin, methyl aurin, and pseudo rosolic acid.

Opinions as to suitability, classified according to groups and chemical composition—Con.

Unfavorable.	Favorable.	Contradictory.
XANTHENE COLORS.		
502. Triethylrhodamin.	**512. Tetrabromofluorescein.**	**504. Hydrochlorid of diethyl-m-amido-phenol-phthalein.**
516. Diiodo fluorescein.	**517. Tetraiodofluorescein.** 520. Tetraiododichlorofluorescein. **521. Tetrabromotetrachloro fluorescein.** **527. Sodium bisulphite compound of coerulein.**	
ACRIDIN COLORS.		
530. Hydrochlorid of diamido-phenyl-dimethyl-acridin. **532. Nitrate of chrysanilin and homologues.**		
ANTHRACENE COLORS.		**563. Dioxy-anthraquinone-b-quinolin.**
INDOPHENOL COLORS.		
572. Tin compound of dimethyl p-amido phenyl-p-oxy-a-naphthylamin. 574. Hydrochlorids of p-phenylene-diamin-p-amidophenol and diamido-diphenylamin.	576. New Gray (composition unknown).	
AZIN COLORS.		
602. Spirit Nigrosines. 614. Amido-naphthyl-naphthazonium chlorid and diamido naphthyl-naphthazonium chlorid.	**593. Phenyl- and tolyl-safranins.** **599. Mixtures of dianilido-amido-trianilido-, and tetraanilido-phenyl-phenazonium chlorids.** **600. Indulins and fluorindins.**	**584. Diamidophenyl and tolyltolazonium chlorids.** **601. Sulphonated indulins.**
OXAZIN COLORS.		
620. Dimethylamido-dioxy-phenazoxonium carboxylate. **639. Chlorid of dimethyl-amido-naphtho-phenoxazonium chlorid.**		
THIAZIN COLORS.		
649. Zinc-double-chlorid of dimethyl-diamido-phenazthionium chlorid. 651. Nitromethylene Blue. **654. Chlorid of dimethyldiamido-toluphenazthionium.**		**650. Chlorid of tetramethyl-diamido-phenazthionium.**
THIOBENZENYL COLORS.		
659. Dehydrothiotoluidin.		
QUINOLIN COLORS.		
667. Quinophthalone.		
SULPHID COLORS.		
670. Sulphohydro derivative of a polythiazin. **675. Thiocatechin S (composition unknown).**		
INDIGO COLORS.		
	692. Indigotin disulphonic acid.	**689. Indigotin.**

PHYSIOLOGICAL ACTION OF COAL-TAR DYES.

SUMMARY OF SYMPTOMS.

A rough summary of the symptoms noted or positively determined to be absent, the number of the deaths produced, and the number of cases in which nothing abnormal was noticed may serve as a convenient guide in considering the detailed statements hereinafter given relative to all the symptoms, clinical data, legislative and other publications, or permissions.

In the following tables are brought together most, if not all, of the recorded observations with respect to the humans and other animals upon which the physiological action of coal-tar dyes has been studied as well as the results of autopsies when recorded. The columns headed "Unfavorable," "Favorable," and "Contradictory" have the same significance as in the preceding table; italicized numbers are those of the permitted colors of Food Inspection Decision No. 76. Asterisked numbers indicate that the dye was administered hypodermically.

EXPERIMENTS ON DOGS.

TABLE I.—*Observations on dogs.*

(Reference to Green Table numbers.)

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Deaths	1; 2; 3; 488 or 490; 574; 639 (2); 649.		16 (2); 55; 70*; 86; 87; 95 (2); 188*; 428 (2); 451 (2); 480*; 601; 650.
Autopsies	3; 488 or 490; 572; 574; 620; 639; 649.	103; 105; 399; 467; 477; 593; *692*.	16; 55; 70*; 87 (2); 45 (2); 457; 480 (2); 601; 650.
Stained skin	1		398.
Stained conjunctivæ	1		398.
Respiration difficulties	1; 3		
Temperature normal	2; 620; 649	467	451; 601.
Temperature high	488 or 490; 3		457.
Temperature low	488 or 490		451.
General depression	425; 649; 675		650.
Weakness	1; 3; 97; 649; 659		16; 483.
Loss of weight	1; 488 or 490; 639		17; 18; 41; 95; 138; 451; 650.
Emaciation	488 or 490; 639		138; 451.
Paralysis of heart			16; 87; 428.
Loss of appetite	2; 3; 97; 425; 530; 649; 659; 667; 670.	240; 394	16; 87; 138; 197; 428; 451; 483; 650.
Aversion to food	649; 3		451.
Colored urine	3; 6; 78; 94; 97; 277; 425; 434; 459; 478; 479; 502; 516; 530; 532; 614; 620; 651; 659; 667; 670.	*4*; 5; 28; 92; 93; 103; 105; 166; 240; 394; 399; 433; 521; 527; 576; 599.	8; 9; 11; 13; 14; 16; 55; 84; 86; 87; 88; 95; 138; 197; 269; 287; 398; 428; 448; 483; 504; 601; 650.
Colorless urine		240	287.
Urine not affected	488 or 490; 649	467; 477	451; 480; 601.
Albuminuria	1; 3; 78; 94; 97; 425; 434; 459; 479; 516; 580; 651; 659; 667.	*4*; 5; 28; 166; 240; 394	8; 9; 11; 17, 18, or 41; 70; 86; 88; 95; 188; 197; 269; 287.
Albuminuria doubtful	78; 478	5	138.
Alkaline urine	670	240	14; 88; 269; 287.
Bloody urine			451.
Phenol in urine			11; 88.
Kidney irritation	1		86.
Bladder irritation	639		650.
Thirst	3		16; 86; 87; 428.
Colored feces	94; 425; 488 or 490; 620	103; 105; 399; 477	87; 88; 398; 480; 601; 650.
Thin stool	425; 502; 614	527	16; 504.
Diarrhea	1; 3; 94; 97; 479; 425; 516; 614; 639; 649; 651; 670.	*4*; 92; 103; 105; 399; 527; 593.	11; 16; 84; 86; 87; 138; 269; 428; 483; 504; 584; 650.
Softened feces			95.
Bloody stool			650.

TABLE I.—*Observations on dogs*—Continued.

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Pus in stool			650.
Stool desires	502		
Vomiting	1; 2; 3; 6; 97; 277; 425; 479; 488 or 490; 516; 532; 574; 614; 639; 649; 670; 675.	399; 433; 467; 576; *698*	11; 16; 86; 87; 95; 197; 269; 428; 448; 451; 483; 650.
Retching	675	28	16; 86.
Salivation	488 or 490; 639; 649; 675		584.
Anemia	488 or 490; 639		
Stupor			601.
Desire to sleep			451.
Catarrh of eyes and nose			451.
Cramps	1; 2		601.
Convulsions	2; 675		
Tremors			87.
Congestion	649		
Paralysis of limbs	1; 675		16; 55; 87; 428; 450.
Destroys coloring matter of blood.	1; 2		
Loss of sight			87; 601.
Loss of hearing			601.
Internal disorders			650.

The following table discloses the results of the autopsies made and reported on dogs.

TABLE II.—*Autopsies on dogs.*

Symptoms.	Unfavorable.	Favorable.	Contradictory.
No change in internal organs.			87.
Nothing abnormal		105; 399; 467; 477; 593	87 III; 457.
No change except whole interior colored red.		103	
LIVER.			
Fatty degeneration	572; 620; 639		601; 650.
Blood-poor	488 or 490		451 (2); 480 II*; 601.
Pale			451 (2).
Swollen	574		
Inflamed	649		650.
Blood-filled	574		
KIDNEYS.			
Congested	3; 639; 649		451 (2); 480* (II).
Filled with decomposed blood corpuscles.	639		451 (2).
Colored			480 (1); 601; 650.
Soft			480* II.
Blood-poor	488 or 490		601.
Blood-filled	574		
Swollen	620; 639	*698*	
Thickened			650.
Inflamed			650.
STOMACH.			
Colored	488 or 490		650.
Catarrh	488 or 490		650.
Inflamed mucous membrane.	574; 649		
Punctured mucous membrane.			601.
Contracted and filled with colored mucous.	639		
LUNGS.			
Filled with blood	574		
Punctured			601.
HEART.			
Swollen	574		
Paralyzed	574		*16* (2); 87 I; 428; 87 II.

TABLE II.—*Autopsies on dogs*—Continued.

Symptoms.	Unfavorable.	Favorable.	Contradictory.
INTESTINES.			
Colored	488 or 490		650.
Catarrh	488 or 490		
COLORED.			
Brain			650.
Fat			650.
Skin			650.
Pleura			
Heart sac			650.
Diaphragm			650.
Œsophagus	488 or 490		650.
All organs colored			480* II.
All organs swollen			480* II.
General congestion	3 (II)		
Peritonitis			70*.
Fat all disappeared	639		
Flabby muscles	639		

In this connection it may be of interest to note that out of 16 dyes producing death when administered through the mouth to dogs, 7 were on the United States market in the summer of 1907. Their Green Table numbers are as follows, the numbers in parentheses indicating the number of makers or importers, out of a possible 12: offering them: 55 (2); 86 (8); 95 (2); 428 (3); 451 (5); 601 (1); 650 (2).

The following table gives the Green Table numbers of coal-tar dyes which on administration to dogs positively did not in certain specific cases produce the particular disturbances recited, although the case of the several colors as a whole is regarded in the literature as "favorable," "unfavorable," or "contradictory," as stated.

TABLE III.—*Observations on dogs showing definite negative results in specific cases grouped under the general verdict of the literature as a whole.*

Symptoms.	Unfavorable.	Favorable.	Contradictory.
Vomiting	614	*4*; 105; 462	95; 601.
Diarrhea		*4*; 105; 462	95; 504.
Albuminuria	6; 97; 277; 434; 532; 614; 651; 650.	*4*; 92; 93; 105; 433; 462; 521; 527; 576.	14; 16; 55; 84; 86; 87; 88; 197; 398; 448; 504; 601.
Sugar in urine		*4*; 394	
Phenol in urine			287.
Loss of weight		*692*	86.

That is, when these dyes were tried on dogs the observers reported in certain specified cases, positively and definitely, the absence of any of the symptoms named.

It is stated of the following colors that they produced no bad effects in certain specified cases: *4*; 9; 65; 86; 105; 188; 197; 240; 457; 572; 593. No bad effect except colored urine: 13, 95. No bad effect except albuminuria: 88, 287. No bad effect except loss of weight: 12.5 per cent (17, 18, or 41).

It is therefore true of all of these coal-tar dyes that there are conditions under which they have been observed not to produce the bad effects as above set forth; but this tabulation must not be taken to mean that these dyes can not, under any conditions whatever, produce untoward results; the reverse is true in most cases.

EXPERIMENTS ON HUMAN BEINGS.

The Green Table numbers of those colors concerning which experimental data are available on humans are as follows: 1; 2; 3; *4;* 6; 9; (17, 18, 41); 55; 65; *85;* 86; 95; 102; 103; 105; 106; *107;* 197; 427; 428; 448; 462; 532; 602; 650.

The symptoms produced are classified in the following list:

Internally administered.

Not poisonous	*4;* 9; 55; 65; *85;* 95; 102; 103;105;106;*107;*448;462
Poor general condition	86; 650
Fever	2
Loss of appetite	2
Vomiting	3; 532; 650
Intestinal irritant	650
Diarrhea	532; 650
Bladder irritant	650
Colored urine	86; 95; 650
Albuminuria	650
Increased micturition	650
Irritant	532
Inflammation	532
Dryness of throat	86

Bad taste in mouth	86
Restlessness	86
Rush of blood to head	86
Vertigo	86; 650
Headache	650
Delirium	650
Twitching of muscles	650
Yellow coloration of skin	3
Yellow-colored mucous membrane.	3
Food colored with it made a family sick	3
Adults withstand	1
Children and weak adults do not withstand	1
Deaths	2; 3
Autopsy	[1] 3

It should be noted that of the 13 dyes here classed as not poisonous to humans all but No. 102 were on the United States market in the summer of 1907, as is shown in the following table:

Number of dealers offering these nonpoisonous colors in 1907.

Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.	Green Table numbers.	Sources offering same.
[1] 4	10	65	2	103	6	107	7
9	1	[1] 85	2	105	1	448	4
55	2	95	2	106	5	462	2

[1] On permitted list, Food Inspection Decision 76.

Symptoms produced by external application of certain colors (Green Table numbers).

Burning	2; 427; 428
Itching	2; 6; 427; 428
Blisters	2; 6
Swelling	427; 428

Dermatitis	*4*
Eczema	(17, 18, or 41); 197; 602
Inflammation	427; 428

It should be noted that No. 86 has been tried on humans and has been found not to produce diarrhea or vomiting.

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[1] Hemorrhagic gastritis.

G. T. 3 has apparently killed a human at 60 mg per kilo and the autopsy disclosed hemorrhagic gastritis. G. T. 448 has been suggested as a possible remedy for Bright's disease. It should also be borne in mind that adults can stand G. T. 1 in doses of 540 to 900 mg daily for a long time, whereas children and weak adults stand that substance only poorly.

Experiments on Small Animals.

Results of experimenting on rabbits with 10 coal-tar dyes whose Green Table numbers are 1, 2, 86, 89, *107*, 427, 448, 504, 517, and 563 have been tabulated as follows:

Death	1; 2; 427*; 563*	Paralysis	427*
Diarrhea	1	Cramps	427*
Colored urine	86	No harm produced	89; *107*; 448; 504; 517
Softened feces	86		

In the case of the numbers marked with an asterisk the color was administered hypodermically. In this connection reference should also be had to the paper of Penzoldt abstracted in Section VIII, page 55.

The four coal-tar dyes 55, 103, 425, and 480 have been tested on guinea pigs and no disturbance was noticed in all, but in the case of 103 occasional thirstiness was observed. No. 448 has been fed to hens without damage, and No. 2 has been recommended as an insecticide, a fungicide, and a mouse poison.

GENERAL STATEMENTS.

The following statements may be of interest before the detailed compilation is read:

1. O. Buss (*Forschungsber. über Lebensmittel, 1896, vol. 2, pp. 163–197, 237*), in a paper entitled "Contributions to the Spectrum Analysis of some Toxic and Pharmacognostically Important Coloring Matters, with Special Consideration to the Ultra-Violet," cites as poisonous the following (Green Table numbers follow in parentheses wherever connection could be satisfactorily established):

Picric Acid	(1)	Metanil Yellow	(95)
Dinitro o- and p-Cresol	(2)	Corallin	(483)
Martius Yellow	(3)	Safranin	(584)
Aurantia	(6)	Methylene Blue	(650)
Fast Yellow	(8)	Iodin Green	(459)
Orange II	(86)	Alizarin Blue S	(563)

As nonpoisonous:

Naphthol Yellow	(4)	Naphthol Green	(398)
Eosin	(512)	Malachite Green	(427, 428)
Erythrosin	(517)	Dahlia	(450, 451)
Anilin Blue	(457)		

As doubtful:

Methyl Violet	(451, 454)	Vesuvin	(197, 201)

On the following Buss is noncommittal:

Auramin	(425)	Magenta	(448)
Biebrich Scarlet	(163)	Aurin	[1](483)
Water blue	(480)	Acid Green	(435)

Naphthol Black of various brands, which seemed to be mixtures.

2. It has been pointed out that the following nine colors are harmless:

Naphthol Yellow	(4)	Wine Green	(?)
Naphthol Brown	(?)	Milling Red	(?)
Chinolin Yellow	(667)	Azo-acid Blue	(36)
Pyrotin RRO	(115)	Fastrose	(?)
Acid Green	(434, 435)		

Most of these have not been examined experimentally, but scientific studies have been made of the poisonous qualities of Azo-Blue and Naphthol Yellow. (*Zts. angew. Chemie, 1896, p. 24.*)

3. Chlopin in his monograph (see p. 75) says:

> On the basis of my personal experience I consider the testing of the action of coal-tar dyes on man not permissible, since such experiments may induce in the subjects of experiment more or less serious symptoms of poisoning, for which in some cases there are no antidotes at our disposal. To such accidents, in my opinion, only the experimenter himself may subject himself, because he knows what he is doing. Preliminary tests of dyes on dogs and other animals afford no guaranty of escape from disagreeable accidents which may take place in the testing of the dye on man. (*Page 111.*)
>
> These data and calculations convincingly prove how erroneous the current opinion is that for the coloring of food substances and beverages only exceedingly small, almost unweighable, quantities of dyes are used. (*Page 113.*)

On page 221 et seq., the following general discussion of this subject is found:

THE MANNER OF ACTION OF POISONOUS DYES ON THE ANIMAL ORGANISM.

> The mechanism and the chemistry of the action of the artificial dyes of the aromatic series on the animal organism remains to the present day, with few exceptions, exceedingly slightly and superficially studied. The same can be said also concerning the pathological and anatomical changes which are induced by these dyes.
>
> More than the others there have been studied in the toxicological respect the dyes belonging to the Nitro group; Picric Acid; Martius Yellow (Dinitro-naphthol potassium), and Saffron substitute (Dinitrocresol potassium.)
>
> According to Kobert, these dyes belong to the poisons acting on the blood. According to the same authority, Methylene Blue, which belongs to the other chemical group of Thiazins, acts similarly.
>
> In the fundamental works on the sanitary investigations for the dyes, by Cazeneuve and Lépine, by Weyl, and by Santori, we find almost no material relating to the explanation of the manner of the action of the dyes. These investigators limiting themselves to a very cursory description of the symptoms of poisoning, and reciting in most general terms the results of autopsy, not even indicating the cause of death. Such, for instance, are the reports of autopsies made by T. Weyl and some other investigators, as quoted above.

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[1] Apparently a purified form of Corallin.

We may expect that more detailed investigations in this respect will be made at the proper time by pharmacologists, since study of the mechanism and chemistry of the action of the poisonous substances on the animal organism is their province; for the hygienist it is quite sufficient merely to establish the fact that a given substance is poisonous or harmful, and he need not go any further. For this reason, in those cases in which I desired to clear up the causes of death of the animals in my experiments, and to record pathological and anatomical changes (although by the terms of the regulations governing this competition, a close study of the action of the dyes, and the ascertainment of the mechanism and chemistry of their action was not required), I called in a person more competent than myself on these questions.

Not counting the duplicates we made five autopsies all told. In all these cases death resulted from paralysis of the heart. The pathological and anatomical changes in all cases, except one, did not present anything specific, and finally reduced themselves to a feebly expressed turbid swelling of the heart and of the liver, a rush of blood to the stomach, and a congestion of the internal organs.

The exception was the autopsy of a dog, which died from Methyl Orange; this dog died with the symptoms of paralysis of a cerebro-spinal nature. This experiment was made twice, and the autopsies of both animals showed hyperæmia in the lowest part of the spinal column, on the border of the anterior and the lateral columns.

As to the symptoms of poisoning not resulting in acute death, here most frequently was observed vomiting, diarrhea, and albumen in the urine, showing disturbance of the functions of the digestive tract, and an affection of the kidneys.

A highly typical picture of poisoning is presented by the sulphid Vidal dyes. They cause rapid, almost instantaneous, deafening of the animal, whereupon the animal falls on one side in convulsions and lies, not moving its body, but convulsively and rapidly twitching its anterior limbs during several minutes. The tongue hangs out of the mouth, a strong secretion of saliva is noted, then vomiting begins, and the dog gradually begins to revive; with difficulty he arises on his front feet and sits down, not being able yet to stand on his posterior extremity, which is in a state of paresis. After a few hours the dog becomes normal. The symptoms of poisoning, just described, are exceedingly similar to the supposed "apoplectic form" of poisoning by hydrogen sulphid, which had been studied on animals by K. Lehmann, and which was observed in persons who inhaled air containing a few per cent of this gas. Air containing 0.1 to 0.3 per cent of hydrogen sulphid kills cats and dogs in 10 minutes.

In our experiments in which were introduced substances containing sodium sulphid, the poisoning must have been caused by hydrogen sulphid which was liberated from the dye by the acid of the gastric juice, and which could cause poisoning also through the stomach and through the respiratory apparatus.

Fortunately Vidal dyes, owing to their repulsive odor, will scarcely find a wide application in coloring food and beverages.

SOME REFLECTIONS REGARDING FURTHER INVESTIGATIONS OF DYES FROM A SANITARY STANDPOINT.

The present investigation, as well as all the investigations of the action of dyes on animals by previous investigators, had for its object the solution of the question in what number there exists among the dyes of the aromatic series dyes which possess poisonous, or more or less pronounced harmful properties (answering essentially the sanitary toxicological question). From the practical point of view such investigations presented and do present the most important interest, inasmuch as they afford a possibility of protecting the public from the use of obviously poisonous and harmful substances, but by such investigations questions of exceedingly sanitary importance are not answered, namely:

1. Ought we to consider as quite harmless those dyes which do not induce pronounced symptoms of poisoning and which are designated herein by the term non-poisonous?

2. Is the usual answer which is given to the hygienist by the defenders of the unrestricted use of the coal-tar dyes for coloring food products and beverages, namely, that in practice the dyes are introduced into the human organism in so small quantities that their properties can be neglected, justified?

To both questions, besides the facts and considerations which I gave above in my investigations, we may reply experimentally in two ways:

(*a*) By prolonged investigations continued over a period of years of the action of very minute quantities on the animal organism, which has so far, owing to the inconveniences of such long experiments, not been done by anybody; and

(*b*) By investigation of the action of small doses of dye on some physiological functions, and first of all on the activity of the digestive organs, which is first of all disturbed upon the introduction of dyes into foods and beverages. The solution of the last question can be best promoted, in my opinion, by experiments made on dogs and by exploratory examination of the body according to the method of Prof. Pawlow. Unfortunately, experiments such as these, owing to the difficulty of the Heidenhain-Pawlow operation, are inaccessible to the majority of investigators.

As a very useful substitute of such investigations may serve observations on the action of dyes on the activity of the digestive juices outside the body of animals.

On my proposition Dr. A. E. Winogradow began in my laboratory experiments on the action of small doses of dyes of the aromatic series on the digestion *in vitro*. Dr. Winogradow so far examined 25 coal-tar dyes in this respect according to the method of Metta and convincingly proved that in insignificant doses coal-tar dyes (from one-half to 4 milligrams) entirely stopped the digestion of albumen by artificial gastric juice. It was found that the capacity to depress the digestion is possessed not only by poisonous dyes, but also by dyes which proved in my experiments on animals nonpoisonous.

It is quite possible, therefore, that an admixture of coal-tar dyes will exert an unfavorable influence on the digestion and assimilation of food prepared from products colored by them. Experimental proof of the last supposition can be given only by experiments on the influence of dyes on the metabolism of substances in animals and man.

COMPLETE DETAILED STATEMENT OF ALL COMBINED DATA.

ABBREVIATIONS OF AUTHORITIES CITED.

The data hereinafter given is brought together as nearly as possible under the Green Table numbers to which it is pertinent. It is believed that the literature has been quite thoroughly searched, and that nothing of substantial importance has escaped recording in this compilation; certainly whatever may have escaped can hardly serve to change the general conclusion to which this compilation leads.

In order to avoid repetition in the following tabulation, "Weyl" is to be understood as referring to the book entitled "The Coal Tar Colors, with Especial Reference to their Injurious Qualities, etc.," by Theodor Weyl, translated by Leffmann and published in Philadelphia, Pa., in 1892.

"Lieber" refers to the book entitled "The Use of Coal Tar Colors in Food Products," by Hugo Lieber, published in New York in 1904.

"Fraenkel" refers to the book entitled "Arzneimittel Synthese," by Dr. Sigmund Fraenkel, published in Berlin in 1906.

"Confectioners List" refers to the Official Circular from the Executive Committee of the National Confectioners' Association of the

United States entitled "Colors in Confectionery" and reprinted, in part, in the book entitled "Food Inspection and Analysis," by Albert E. Leach, published in New York in 1906, pages 630–634.

"Resolutions of Swiss Analytical Chemists" refers to these resolutions as published in Zeitschrift für Nahrungsmittel Untersuchung und Hygiene, 1891, page 293.

"Schacherl" refers to Schacherl's publication entitled "Die Zulaessigkeit Kuenstlicher Farbstoffe zum Färben von Lebensmitteln," published in Vol. III, pages 1041–1048, of the Report of the Fifth International Congress of Applied Chemistry held in Berlin June 2 to 8, 1903.

"Chlopin" refers to Chlopin's monograph published in Russian and entitled "Coal Tar Dyes. Classification, properties, and action of artificial dyes on the animal organism, etc.," published at Dorpat in 1903, or to the abstract of Chlopin's paper printed at page 169–172 of Vol. IV of the Report of the Fifth International Congress of Applied Chemistry held in Berlin, 1903.

"Canton of Tessin" refers to the publications of the Tessin regulations published in 1897 in Zeitschrift für Untersuchung der Nahrungs und Genussmittel, page 414.

Whenever possible the doses administered have been given in milligrams per kilo and grains per 100 pounds of body weight of animal. In the case of the tabulations taken from Chlopin's monograph this was not done; but in order to render such comparative data easily available factors have been placed at the head of each tabulation; for example, under G. T. 6 (1 gram=106 mg=74.2 grains), which means that each gram administered amounts to 106 mg per kilo or 74.2 grains per 100 pounds of body weight of animal; by multiplying the doses given by either of the factors the corresponding comparative information is obtained.

In addition to the 106 Green Table numbers that have been examined physiologically, there are reported the results of the physiological examination of 8 coal-tar colors not in the Green Tables, of which 3 are said to be nonpoisonous, 3 are called poisonous, 1 is called harmful, and the last is said to be "not quite harmless." These 8 dyes are not included in this compilation.

TABULATION BY GREEN TABLE NUMBERS OF PHYSIOLOGICAL AND OTHER DATA.

G. T. 1.

Trade names.—Picric acid; carbazotic acid.
Scientific name.—Symmetrical trinitrophenol.
Discovered.—1771.
Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 30*): "The injurious character of picric acid has long been known."
3. "In Germany its employment for coloring food is forbidden by the imperial enactment of 1888, on account of its poisonous character." (*pp. 68–71.*)
4. "The foregoing statements show that while the acid must be considered poisonous, its injurious character is far less than has generally been assumed, nevertheless, the legal prohibition of its use as a coloring matter for food or drink is just." (*p. 71.*)
5. "Erb gave a rabbit weighing 1,700 grams, 0.06 gram of potassium picrate (24.5 grains per 100 pounds) daily for 90 days; slight loss of weight and occasional diarrhea were noted, but nothing more serious." (*p. 69.*)
6. A rabbit weighing 2,065 grams died at the end of 19 days, after having taken 2.52 grams of the substance, or 854 grains per 100 pounds body weight; number of doses not stated. (*p. 69.*)
7. Weyl's experiment on a dog, weight not given: April 21–26, 0.24 gram (3.7 grains) sodium picrate daily; April 28–May 9, 0.36 gram (5.5 grains) daily; total, 5.76 grams (8.9 grains) sodium picrate; no serious disturbance; May 13, 1.2 grams (18½ grains) sodium picrate at one dose; weakness marked, diarrhea and dyspnea next day; May 14, 0.6 gram (9¼ grains) caused vomiting; evening of same day, 0.36 gram (5.6 grains) given; May 15, animal lively; 0.24 gram (3.7 grains) again given, and on evening of same day 0.72 gram (11.2 grains); May 16, marked weakness of animal, and 0.16 gram (2.5 grains) given, causing vomiting; May 17, 0.17 gram (2.6 grains) given; May 18 and 19, animal definitely recovered, and aside from strong yellow tinge of the conjunctiva and skin, no abnormal conditions manifest. Animal died May 20 after receiving 1.32 grams (20.4 grains) potassium picrate. Weyl concludes, therefore, that dogs are resistant to this substance, notwithstanding the prostration and the blood disorganization.
8. Weyl summarizes the effect on humans from therapeutic and poisoning cases to the effect that daily doses of from 0.54 to 0.90 gram (8.3 to 13.8 grains) of potassium picrate are easily borne by healthy adults for a considerable time; children and weak adults bear picric acid badly. (*p. 70.*)
9. "Picric acid * * * is poisonous * * *." (*p. 96.*)
10. **Fraenkel** (*p. 572*): "On the other hand, this substance is not usable for internal administration on account of its decomposing the red blood corpuscles, and of its energetic cramp production, as well as on account of its disturbance of the kidneys, and the ultimate paralysis of the respiratory centers; nevertheless, picric acid is not to be considered a violent poison * * *."
11. **Schacherl** (*p. 1044*): "Picric acid * * * (is), according to numerous statements in the literature, poisonous even in small doses, and (is) therefore unqualifiedly to be declared as unpermissible."
12. **Lieber** (*p. 16*), where it is stated to be forbidden by the German law, and is also otherwise substantially the same as Weyl above quoted.
13. Resolutions of the Society of Swiss Analytical Chemists, September, 1891: "The following are to be regarded as coloring matters harmful to health: * * * picric acid * * *."
14. Prohibited by the Belgian law of June 17, 1891.
15. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Picric acid is poisonous. Rabbits can stand daily 10 milligrams of a green containing picric acid, but not 20 milligrams. Their death is accompanied by paralysis."
16. **Buss** lists it as poisonous.

G. T. 2.

Trade names.—Victoria Yellow; Victoria Orange; Golden Yellow; Saffron Substitute; Anilin Orange; Di-nitro-Cresol.

Scientific name.—Di-nitro-o-and-p-cresol.

Shade.—Yellow. Not offered.

Discovered.—1869.

Used for coloring butter, liqueurs, etc.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Fraenkel** (*p. 572*): "On the other hand dinitro-cresol is much more intensely poisonous (than picric acid), which is probably caused by its greater solubility in water."
3. **Schacherl** (*p. 1044*): * * * Dinitro-cresol [is], according to numerous statements in the literature, poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
4. Resolutions of the Society of Swiss Analytical Chemists, September, 1891: "The following are to be regarded as coloring matters harmful to health * * * Dinitro-cresol * * *."
5. Forbidden by the Canton of Tessin.
6. **Weyl** (*p. 31*): "I have shown the same (poisonous nature) for Dinitro-cresol (Saffron Substitute). (See *Zts. angew. Chem.*, 1888, No. 12, for confirmation of my results by Gerlach.)"
7. "The reverse is the case with the poisonous dinitro-cresol (Saffron Substitute)." (*p. 55.*)
8. **Weyl** describes experiments with this compound. (*pp. 71–85.*)
9. Fourteen rabbits were experimented on, of which 13 died. Amounts administered in the fatal cases per 100 pounds body weight were (*p. 74*):

Grains.	Grains.
189	175
175	168
175	175
175	168
175	168
175	175
175	

Of 12 experiments on dogs, 5 receiving the color by the mouth and 7 hypodermically, 3 cases resulted fatally; the fatal case by the mouth requiring 38½ grains per 100 pounds body weight; the 2 fatal cases hypodermically represented 11 and 20 grains per 100 pounds body weight, respectively, although 140, 38.5, 31.6, and 35 grains per 100 pounds body weight by the mouth were borne without fatal effect; and 24½, 11.9, 9.8, and 4.9 grains per 100 pounds body weight, hypodermically, were also borne without fatal effect (*p. 75*).

10. **Weyl** (*p. 96*): "* * * Dinitro-cresol * * * are [is] poisonous; * * *"
11. Prohibited by the Belgian law of June 17, 1891.
12. (*Zts. Nahr. Genussm., 1892, p. 353*): Recommended as an insecticide, 1500 being sufficient for all ordinary purposes. One milligram is sufficient to kill a mouse; 2 milligrams recommended for killing mice.
13. **Weyl** (*Handbuch der Hygiene*): For humans, the fatal dose, when administered by the stomach, appears to be 60 milligrams per kilo body weight, or 43 grains per 100 pounds.

14. "The president of the Council of Oppeln forbids on April 19, 1899, the use of Saffron Surrogate for coloring food products."
15. Lewin (*Lehrbuch der Toxikologie, 1897, p. 232*): "Saffron Surrogate * * *, which is used for coloring foodstuffs, is poisonous. It appears to attack the coloring matter of the blood, and produces, in the case of dogs, vomiting, cramps, and convulsions. * * * Feathers colored with Saffron Surrogate cause burning and itching, and finally blisters on the hands of the women working with them; the faces were also similarly affected, and this was accompanied by loss of appetite and fever."
16. Buss lists it as poisonous.

G. T. 3.

Trade names.—Martius Yellow; Naphthol Yellow; Naphthylene Yellow; Naphthylamin Yellow; Manchester Yellow; Golden Yellow; Saffron Yellow; Jaune d'Or; Jaune Naphthol.

Scientific name.—Dinitro-alpha-naphthol.

Discovered.—1864.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of Martius Yellow * * *."
3. "This body (Chamber of Commerce at Sonneberg) recommends for the preparation of children's toys three colors, the poisonous character of which I can demonstrate. These are Martius Yellow * * *." (*p. 34.*)
4. "For instance, for preliminary researches, dogs and rabbits have value for chemical reasons. The conclusions derived from such experiments must be accepted with great deliberation, since it happens that rabbits will bear without injury doses which will seriously, nay, even fatally, act upon the dog, as I have already shown to be the case with Martius Yellow." (*p. 56.*)
5. Where two experiments by Cazeneuve and Lépine are referred to, in which diarrhea, vomiting, and albuminuria were produced by this substance. (*pp. 85–89.*)
6. Weyl's own experiments on 4 dogs showed weakness, vomiting, diarrhea, and albuminuria resulting from the use of this color; the amounts of color administered per kilogram of body weight were 73, 17.5, 17.5, and 11.3 milligrams, which amount to 51, 12, 12, and 8 grains, respectively, per 100 pounds of body weight. (*p. 87.*)
7. "Martius Yellow, therefore, belongs to the injurious colors. As a coloring matter for food and drink its use should be wholly prohibited." (*p. 89.*)
8. "* * * and Martius Yellow are poisonous; * * *." (*p. 96.*)
9. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): "Like Saffron Surrogate, it is poisonous. In an experiment on myself, using large doses, I noticed among others the general yellow coloration of the skin. In a poisoning resulting fatally after 5 hours with Martius Yellow, vomiting, yellow coloration of the skin and mucous membranes were observed; whereas the autopsy revealed, among other things, hemorrhagic gastritis. (Jacobson, Hosp. Tid., 1893, p. 765.)"

10. "Such small amounts as are used for the coloring of pastry are said to be non-poisonous. (*Vitali boll. chim. farm.*, 1893, p. 738.)" (*p. 231.*)
11. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*) say: I. "A dog received 71 milligrams per kilogram of body weight, or 50 grains per 100 pounds daily. On the second day diarrhea and vomiting ensued; loss of appetite except for milk. Thereafter it experienced difficulties in breathing; suffered albuminuria; its urine was colored; it died on the sixth day. The autopsy disclosed considerable congestion. II. A dog weighing 22 kilos received 400 milligrams (19 milligrams per kilo or 13 grains per 100 pounds); this caused a yellow vomit; next day it received 500 milligrams (27 milligrams per kilo or 17 grains per 100 pounds), which caused violent diarrhea, fever, thirst, disinclination for all food. The animal was killed; the autopsy showed badly congested kidneys."
12. Prohibited by the Belgian law of June 17, 1891.
13. **Schacherl** (*p. 1044*): "* * * Martius Yellow * * * [is], according to numerous statements in the literature, poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
14. Prohibited by law in Italy. (See *Lieber, p. 24.*)
15. **Fraenkel** (*p. 572*): "This substance also shows poisonous properties, although it is less poisonous than dinitro-cresol."
16. *Resolutions of the Society of Swiss Analytical Chemists, September, 1891:* "The following are to be regarded as coloring matters harmful to health * * * Martius Yellow * * *."
17. Forbidden by the Canton of Tessin.
18. **Dietrich** (*Zts. Nahr. Genussm., 1902, v. 5, p. 364*): "A lot of groats, after eating which a family became sick, was found to be free from ordinary poisons, but had been colored with Martius Yellow."
19. **Buss** lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahrs. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 4.

Trade names.—Sulphur Yellow; Sulphonaphthol Acid Yellow; Succinic; Solid Yellow; Saffron Yellow; Jaune Acide C.; Jaune Acide; Fast Yellow; Citronin; Anilin Yellow; Acid Yellow S.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Yellow SLOZ; Naphthol Yellow S; Naphthol Yellow; Naphthol Yellow L; Yellow F Y; Lemon Yellow.

Scientific name.—Dinitro-alpha-naphthol-beta-monosulphonic acid.

Discovered and patented.—1879.

Shade.—Yellow. Offered by 10 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*): "A dog received each day for 5 days 32 milligrams per kilogram of body weight, or 23 grains per 100 pounds; for the 10 days next succeeding it received four times that amount, that is, 133 milligrams per kilogram of body weight, or 93 grains

2. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*)—Continued. per 100 pounds; for the 10 days next succeeding it received daily twice the last amount, or 266 milligrams per kilogram of body weight, that is, 186 grains per 100 pounds. It received altogether in the 25 days 62½ grams, or 964 grains. There was no vomiting, no diarrhea, and no albumen in the urine at any time."
3. **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs: Naphthol Yellow S. * * *."

   Weyl describes his own experiments on 3 dogs, giving them, respectively, 417, 34, and 100 milligrams per kilo body weight, or per 100 pounds 292, 24, and 70 grains, respectively. Whether the color was administered by the mouth, or injected subcutaneously, all bodily functions appeared to remain normal, and it was only in the case of repeated doses of 417 milligrams per kilogram of body weight, or 292 grains per 100 pounds, that albuminuria appeared. (*pp. 89–92.*)
4. "Only the sulphonated colors Naphthol Yellow * * * are harmless and applicable to the coloring of food and drink." (*p. 96.*)
5. Permitted by the laws of Austria.
6. Permitted by the law of Italy.
7. Permitted by the law of France.
8. **Schacherl** (*p. 1044*): "* * * Naphthol Yellow S * * * possesses no poisonous properties."
9. **Fraenkel** (*p. 572*): "Naphthol Yellow S is an entirely nonpoisonous substance."
10. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, p. 900*): One hundred milligrams per kilogram of body weight for the initial administration, and subsequent administrations increased geometrically. After the second administration intermittent diarrhea resulted, emphasized by increased amounts with no albumin or sugar in the urine; continued for 14 administrations; so that in 14 administrations 147.58 grams of color had been given; the initial dose is 70 grains per 100 pounds of body weight, and the average daily dose of the total administered is 394 grains per 100 pounds of body weight. Urine only slightly yellow colored after small doses, but red after larger doses.
11. **Lieber** (*p. 143*): A dog received 36 milligrams per kilogram of body weight, or 25.2 grains per 100 pounds once a day seven times every other day; during the whole period the dog was apparently in good condition with no bad effects from the color.
12. **Buss** lists it as nonpoisonous.
13. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*): Three chronic invalids received daily from 2 to 4 grams of the dye in cochets; except slight colic and diarrhea nothing abnormal.

DOUBTFUL.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Acid Yellow S is said to be able to produce dermatitis on frequent contact therewith."
2. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 5.

Trade name.—Brilliant Yellow; Naphthol Yellow S or RS.
Scientific name.—Dinitro-alpha-naphthol-alpha-monosulphonic acid.
Discovered and patented.—1884.
Shade.—Yellow. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*pp. 92–94*): Describes experiments on 2 dogs, in which the initial dose was 532 milligrams by the mouth, and 17 milligrams hypodermically, per kilogram of body weight, respectively, 372 and 12 grains per 100 pounds of body weight; in both cases the urine was colored, in the second case traces of albuminuria resulted. In the first case the albuminuria was doubtful.

3. "Brilliant Yellow is not poisonous, even in large doses, when administered by the stomach * * * the albuminuria was very slight. (*p. 94.*)
4. "Only the sulphonated colors * * * Brilliant Yellow, are harmless, and applicable to the coloring of food and drink." (*p. 96.*)
5. **Schacherl** (*p. 1044*): " * * * Brilliant Yellow * * * possesses no poisonous properties."
6. **Fraenkel** (*p. 572*): "For the same reason * * * Brilliant Yellow * * * is without effect."

G. T. 6.

Trade names.—Aurantia; Nitrodiphenylamin; Imperial Yellow; Kaiser Yellow.

Scientific name.—Hexanitro-diphenylamin.

Discovered.—1873.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Weyl** (*p. 96*): " * * * Aurantia suspicious."
2. **Schacherl** (*p. 1044*): " * * * Aurantia [is] according to numerous statements in the literature poisonous even in small doses, and [is] therefore unqualifiedly to be declared as unpermissible."
3. **Chlopin** (*p. 116*) as results of his experiments considers it injurious. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=106 mg=74.2 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 12	1	9.4	450	Before experiment urine and dog normal.
13	2	....	425	Vomited several times; urine orange, and no albumen.
14	3	....	400	Repeated vomiting; urine dark brown, acid; no albumen; dog eats.
15	3	....	390	No vomiting; urine almost black; acid; no albumen.
18	3	....	410	No vomiting; urine dark brown, acid; no albumen.
19	3	....	400	Vomiting; urine almost black; acid; no albumen.
20	3	....	....	No vomiting; urine almost black, acid; no albumen.
21	....	....	425	Same; urine chocolate brown, acid; no albumen; general condition normal.
22–26	....	9.3	....	Gradually color of urine becomes normal; in every other respect dog is well.
Total.	18			

4. **Fraenkel** (*p. 573*): "The coloring matter called Aurantia, which is a salt of hexanitro-diphenylamin, appears to be poisonous on account of the nitro groups, which is on the other hand denied by a few observers."
5. *Resolutions of the Swiss Analytical Chemists, September, 1891:* "The following are to be regarded as coloring matters harmful to health * * * Aurantia * * *."
6. Forbidden by the Canton of Tessin.
7. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Aurantia has a poisonous action. After wearing gloves for 8 hours made with so-called dogskin, which were colored with Aurantia, a man suffered confluent blisters, accompanied by itching. The workmen with this material get blisters on the face and on the hands. Perspiration increases the tendency to such blisters."
8. **Buss** lists it as poisonous.

G. T. 8.

Trade names.—Acid Yellow; Fast Yellow G; Acid Yellow G; Fast Yellow; Fast Yellow extra; Jaune Acide; New Yellow L.

Names under which it was offered on the United States market as a food color in 1907.—Fast Yellow Y; Fast Yellow G; Acid Yellow G pat.; Fast Yellow 053.

Scientific name.—Amidoazobenzene-disulphonate with some sodium amidoazobenzene-monosulphonate.

Discovered.—1878.

Shade.—Yellow. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., April 27, 1886, p. 643*), where it is classified among the "nontoxic" colors.
3. **Fraenkel** (*p. 575*), where it is stated to be nonpoisonous.
4. Permitted by the law of Austria.

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous to human beings. (?)"
2. **Chlopin** (*p. 151*) considers that the work of others makes this a suspicious color. His own experimental data are as follows:

Experimental data by Chlopin.

G. T. 8 AND 9.

[1 gram=152 mg=106 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Mar. 11-13	....	....	310	Dog normal; color urine normal; acid; no albumen.
14	1	6.6	....	Do.
15	....	....	....	Do.
16	....	....	....	Urine greenish brown; reddens with H_2SO_4 and HCl; no albumen.
17	....	....	305	Color same; traces of albumen.
18	....	....	380	Color less intense; trace of albumen.
19	3	6.4	320	Do.
20	....	....	280	Urine greenish brown; albumen gone; dog is lively.
21	....	6.5	292	Everything normal.
Total.....	4			

Conclusion: "Suspicious."

3. **Buss** (*Forschungsber. über Lebensmittel, 1896, Vol. III, p. 173*): Is regarded as poisonous.
4. **Kobert** (*Lehrbuch der Intoxicationen, 1893, p. 335*): Listed as poisonous.
5. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says "produces eczema," and cites *Deutsche Med. Wochenschr., 1891, p. 45.*

G. T. 9.

Trade names.—Fast Yellow R; Fast Yellow; Yellow W.

Name under which it was offered on the United States market as a food color in 1907.—Fast Yellow 034.

Scientific name.—Sodium salt of amidoazotoluene-disulphonic acid.

Discovered and patented.—1878.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): " * * * not poisonous to human beings and dogs * * * Solid Yellow."
3. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 1167–1169*): A. A dog received 42 milligrams per kilogram of body weight, or 29 grains per 100 pounds, for 5 days; thereupon received four times that amount for 5 days, or 168 milligrams per kilogram body weight, or 117 grains per 100 pounds; for the 10 days next succeeding it received daily twice the last dose, or 336 milligrams per kilogram of body weight, that is, 235 grains per 100 pounds; it then received in 1 day 20 times the original dose, or 840 milligrams per kilogram body weight, that is, 596 grains per 100 pounds, and during the entire period nothing abnormal was noticed. B. Three chronic invalids received from 2 to 4 grains of the dye daily; except colic without diarrhea nothing abnormal. They concluded that this dye is no more harmful than Naphthol Yellow S (G. T. 4).
4. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, well or sick.

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous to human beings. (?)"
2. **Chlopin** (*p. 151*): Where he considers that the work of others makes this a suspicious color. For his experimental data thereon see table under G. T. 8; Chlopin's chemical description of the dye used applies to both G. T. 8 and 9.
3. **Kobert** (*Lehrbuch der Intoxicationen, 1893, p. 336*): Listed as poisonous.

G. T. 11.

Trade names.—Sudan I; Carminaph.

Names under which it was offered on the United States market as a food color in 1907.—Oil Orange 7078; Cerasin Orange I.

Scientific name.—Benzene-azo-betanaphthol.

Discovered.—1883.

Shade.—Orange Yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Weyl** (*p. 115*): "Nonpoisonous * * * Soudan I * * *."
2. "Other Azo-colors, * * * for instance Soudan I * * * are entirely nonpoisonous." (*p. 148.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 119*): Dog received 18 grams in 6 doses in 17 days, and remained under observation 5 days longer; 4 doses of 168 milligrams per kilogram body weight (118 grains per 100 pounds) and 2 doses of 420 milligrams per kilogram body weight (294 grains per 100 pounds) produced colored urine, phenol in urine, vomiting and distinct albuminuria beginning with the third dose. From the foregoing Weyl concludes as follows: "The color in the doses administered is not entirely harmless, since a limited albuminuria seems to be brought about."
3. **Fraenkel** (*p. 576*): "It is not wholly harmless, since this coloring matter seems to produce a slight albuminuria."

G. T. 13.

Trade names.—Ponceau 4 G B; Crocein Orange; Brilliant Orange; Orange G R X.

Names under which it was offered on the United States market as a food color in 1907.—Crocein Orange 10234; Crocein Orange; Crocein Orange G; Ponceau 4 G B.

Scientific name.—Anilin-azo-betanaphthol-monosulphonic acid.

Discovered.—1878.

Shade.—Orange Yellow. Offered by 6 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Ponceau 4 G B * * *."
3. Experiment on dog in doses of 161 milligrams per kilogram body weight; that is, 113 grains per 100 pounds body weight, apparently produced no disturbance aside from coloring of the urine. (*p. 124.*)
4. **Weyl** (*p. 148*): "Other Azo-colors * * * for instance * * * New Coccin * * * are entirely nonpoisonous."
5. Weyl's conclusion reads as follows: "This color can be regarded as nonpoisonous."
6. **Fraenkel** (*p. 577*): "Ponceau 4 G B can be regarded as nonpoisonous."

UNFAVORABLE.

1. Excluded by law of Austria.

G. T. 14.

Trade names.—Orange G; Orange G G.

Names under which it was offered on the United States market as a food color in 1907.—Orange G G crystals; Orange G.

Scientific name.—Anilin-azo-betanaphthol-disulphonic acid G.

Discovered and patented.—1878.

Shade.—Orange Yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. Chlopin examined this color, and his experimental data are as follows:

Experimental data by Chlopin.

No. 1 (*p. 123*).

[1 gram=73 mg=51 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 4	1	13.7	525	Nothing abnormal; no albumen.
5			600	Urine light chocolate brown, acid; no albumen.
6	2		990	Urine clear, yellow, blackish sheen; no albumen.
7			550	Urine clear, dark brown; no albumen; faintly alkaline.
8			680	Do.
9			730	Nothing abnormal.
10			755	Do.
Total .	3			

No. 2 (*p. 124*).

[1 gram=119 mg=103 grains.]

1901.				
Nov. 8		8.4	315	Nothing abnormal; no albumen.
12	3			Do.
13-14			370	Urine orange in color, acid, no albumen; in other respects nothing abnormal.
15	3			Do.
16	3		250	Do.
17	3		320	Do.
18	3		210	Do.
20			225	Do.
21			295	Do.
Total .	15			

Conclusion: Nonpoisonous.

UNFAVORABLE.

1. Excluded by Austrian law.

G. T. 15.

Trade names.—Ponceau 2 G; Orange R.

Scientific name.—Sodium salt of benzene-azo-beta-naphthol-disulphonic acid R.

Shade.—Bright Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

UNFAVORABLE.

1. Excluded under Austrian law.
2. Excluded under Swiss laws.

G. T. 16.

Trade names.—Butter Yellow; Oil Yellow.

Scientific name.—Dimethyl-amido-azo-benzene.

Discovered.—1875.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Weyl** (*p. 31*): "Butter Yellow produces no disturbance in rabbits."

UNFAVORABLE.

1. **Chlopin** (*p. 138*): Where as a result of his own experiménts he considers it poisonous. His experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=159 mg=111 grains.]

Date.	Dose.	Weight.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	
May 6	3	6.3	Before experiment urine normal; after a few hours vomiting; urine not collected.
7-8			No vomiting; eats poorly; more tired than usual.
9-15			Dog gradually becomes normal.
16	8		Vomiting; loss appetite.
17			Repeated vomiting.
18			Vomiting continues.[1]

No. 2.

[1 gram=96 mg=67 grains.]

1903.			
Feb. 5	[2] 3	10.4	Before experiment dog quite normal; acid; no albumen.
6			Vomiting during night; dog does not eat, but drinks; urine more yellow than normal; acid; no albumen; in the evening the dog walked and drank water.
7			Died during the night; thin excreta in kennel; autopsy showed paralysis of heart as causing death.

No. 3.

[1 gram=167 mg=117 grains.]

1903.			
Feb. 8	[2] 2	6	Five hours after administration vomiting and involuntary thin feces.
9			Dog does not eat; drinks much; weak; urine dark yellow; no vomiting.
10			Do.
11			Dog lies in cage; moves slowly when allowed out of cage; in the evening retching.
12			Dog is still weak, but general condition somewhat better; urine strikingly colored; acid; no albumen.
13			Began to eat; no albumen.
14-16			General condition improving and almost normal; lassitude continues more than usual; urine normal color; acid; no albumen.
17	[3] 2		In the evening repeated vomiting; loss of apetite.
18	2		No vomiting; in the evening bothersome; does not eat.
19		5.7	Stools normal; striking disturbance of movements; paralysis of legs; must spread hind legs to stand; when pushed not only falls, but turns on his back; sight and hearing are normal; dog died at night.

[1] This dog ran away. [2] Grueblor's make. [3] Berlin make.

G. T. 17, 18, 41.

17. *Trade names.*—Chrysoidin Y; Chrysoidin crystals.

Name under which it was offered on the United States market as a food color in 1907.—Chrysoidin Y.

Scientific name.—Anilin-azo-meta-phenylene-diamin.

Discovered.—1875.

Shade.—Orange. Offered by 2 out of 12 sources.

18. *Trade names.*—Chrysoidin R; Cerotin Orange; C extra; Gold Orange for cotton.

Name under which it was offered on the United States market as a food color in 1907.—Chrysoidin R.

Scientific name.—Anilin-azo-meta-tolylene-diamin.

Shade.—Yellow brown. Offered by 1 out of 12 sources.

41. *Trade name.*—Chrysoidin R.

Scientific name.—Hydrochlorid of toluene-azo-meta-tolylene-diamin.

Discovered.—1876.

Shade.—Orange Brown.

(NOTE.—It has not been possible accurately to differentiate in the literature as to whether Nos. 17, 18, or 41, or all three, were referred to.)

FAVORABLE.

1. WEYL (*p. 115*): "Nonpoisonous * * * Chrysoidin * * *."
2. Permitted by the law of Italy.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. WEYL (*p. 126*): Experiment on dog, giving him 113 milligrams per kilogram body weight, or 79 grains per 100 pounds body weight, produced a slight albuminuria.

   In a second experiment a dog receiving "1 grain" ("grain" is evidently a misprint for "gram" and will be so treated), that is, 105 milligrams per kilogram body weight, or 74 grains per 100 pounds body weight, daily for one month, did not produce albuminuria, but caused a loss of body weight of about 12.5 per cent. A third dog receiving about 2 milligrams per kilogram body weight, or about 1.5 grains per 100 pounds body weight subcutaneously, suffered a loss of 20 per cent of its body weight in 23 days; dose repeated 24 days after and animal kept under observation seven days longer when animal was normal.
3. Weyl in summarizing experiments on these three dogs concludes as follows: "Chrysoidin produces, according to my investigations, a slight albuminuria, and notable reduction in body weight, but further disturbance has not been noted." (*p. 127.*)
4. FRAENKEL (*p. 577*): "The above-mentioned Chrysoidin * * * produces a slight albuminuria, and a notable decrease of body weight, and produces factory eczema."
5. LEWIN (*Lehrbuch der Toxikologie, 1897, p. 231*): "Produces eczema," and cites *Deutsche Med. Wochenschr., 1891, p. 45.*

G. T. 28.

Trade name.—Archil Substitute V.

Scientific name.—Sodium salt of para-nitrobenzene-azo-alpha-naphthylamin-para-sulphonic acid.

Discovered and patented.—1878.

Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Archil Substitute * * *."
   Describing experiments on three dogs, as follows: A. 430 milligrams per kilogram body weight, or 301 grains per 100 pounds body weight, on each of two successive days, and double the dose on the fourth day, producing no vomiting, but a tendency to vomit, a slight albuminuria and colored urine. B. 182 milligrams per kilogram body weight, or 127 grains per 100 pounds body weight, administered daily for one month; results similar to foregoing, but no colored urine. C. 105 milligrams per kilogram body weight, or 116 grains per 100 pounds body weight administered subcutaneously, produced only slight albuminuria, and no reduction in body weight. (*p. 125.*)

G. T. 43.

Trade names.—Orange GT; Orange RN; Orange O; Orange N.

Scientific name.—Sodium salt of toluene-azo-beta-naphthol-sulphonic acid.

Discovered.—1879.

Shade.—Orange. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

UNFAVORABLE.

1. Excluded by Austrian law.
2. Excluded by Swiss laws.

G. T. 55.

Trade names.—Ponceau R; Ponceau 2 R; Ponceau G and GR; Xylidin Red; Xylidin Scarlet.

Names under which it was offered on the United States market as a food color in 1907.—Scarlet; Orange R.

Scientific name.—Sodium salt of xylene-azo-beta-naphthol-disulphonic acid.

Shade.—Scarlet. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): "According to Cazeneuve and Lépine's experiments the following are not poisonous to human beings and dogs * * * Ponceau R * * *."
3. "Ponceau R (Ponceau 2 R, Xylidin Red, Xylidin Ponceau), not poisonous to dogs neither by administration by stomach nor injection into blood." (*p. 115.*)
4. "Other Azo colors * * * Xylidin Red * * * are entirely nonpoisonous." (*p. 148.*)
5. **Fraenkel** (*p. 575*): "That the monazo coloring matters examined by Cazeneuve and Lépine, as already above stated, were nonpoisonous, can be easily explained by the constitution of these substances. These two investigators examined * * * Ponceau R * * *."

6. Lieber (*p. 140*): A guinea pig received 310 milligrams per kilogram body weight or 217 grains per 100 pounds body weight, once a day six times every other day; the appetite appeared to remain good, and no disturbances were noted.
7. Permitted by Austrian law.
8. Permitted by Swiss laws.
9. Cazeneuve and Lépine (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man well or sick.
10. Chlopin (*p. 150*) classes it as nonpoisonous on his own experiments. His experimental data are as follows:

Experimental data by Chlopin.

[1 gram=141 mg=99 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	cc.	
May 3	2	7.1	370	Dog and urine normal and no albumen.
4	....	....	351	Urine rose-colored; no albumen.
5	2	....	420	Color normal; no albumen.
6	....	....	290	Rose-colored urine; no albumen.
7	2	....	359	Normal color; no albumen.
8	....	....	360	Rose-colored; no albumen.
9	....	....	330	Color and composition normal.
Total.	6			

UNFAVORABLE.

1. Prohibited by the ordinance of the police commissioner in France. (See *Lieber, p. 30.*)
2. Meyer (*J. Amer. Chem. Soc. 1907, v. 29, pp. 900–901*): The dog experimented on showed signs of paralysis on the morning of the seventh day at 8 o'clock, and died at 10.40 a. m., after having received a total of 32 grams of color, of which 16 had been given on the last day. The initial dose was 70 grains per 100 pounds body weight; the total weight of color was 5,818 milligrams per kilogram body weight, or 4,073 grains per 100 pounds; the average daily dose was therefore 582 grains per 100 pounds, or 831 milligrams per kilogram body weight.

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 65.

Trade names.—Fast Red B; Bordeaux B; Bordeaux BL; Bordeaux R extra.

Names under which it was offered on the United States market as a food color in 1907.—Bordeaux B; Claret Red.

Scientific name.—Alphanaphthylamin-azo-betanaphthol-disulphonic acid.

Discovered.—1878.

Shade.—Red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Bordeaux Red (Fast Red B) not poisonous to human beings."
3. "Other Azo colors * * * Fast Red B * * * are entirely nonpoisonous." (*p. 148.*)
4. **Fraenkel** (*p. 575*): "That the monoazo coloring matters examined by Cazeneuve and Lépine, as already above stated, are nonpoisonous, can be easily explained by the constitution of these substances. These two investigators examined * * * Bordeaux B."
5. **Arloing and Cazeneuve** (*Archives de physiologie, 1887, pp. 356–393*): As a result of this work, which is divided into three parts—(1) Stating the effect of direct introduction of the color into the circulation; (2) intravenous injections; (3) comparing the effects of injections of color and of salt; and (4) feeding by the mouth—these investigators conclude that these coloring matters are toxic only in extremely large doses; that when given to dogs with their food that no inconvenience of any kind results; this is based upon experiments on three dogs, covering 145 days, where each dog received per kilogram of initial body weight in the first case 20,307 milligrams, or 14,213 grains per 100 pounds initial body weight; in the second case, 29,590 milligrams, or 20,713 grains per 100 pounds initial body weight; and in the third case, 28,154 milligrams, or 19,758 grains per 100 pounds initial body weight. Per day this means 98 grains per 100 pounds initial body weight in the first case; in the second case, 143 grains per 100 pounds initial body weight; and in the third case, 137 grains per 100 pounds initial body weight.
6. **Cazeneuve** (*Arch. gén. de méd., 1886, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
7. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man well or sick.

G. T. 70.

Trade name.—Azarin S.

Scientific name.—Ammonium bisulphite compound of dichlorophenol-azo-beta-naphthol.

Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 115*): "Nonpoisonous * * * Azarin S * * *."
3. Experiments on five dogs; three fed by the mouth; two treated hypodermically, of which latter one died. The first dog received 1,367 milligrams per kilogram body weight in 25 days; that is, 54.7 kilograms per day on the average, or a total of 957 grains per 100 pounds of body weight; that is, 38 grains per 100 pounds body weight per day. The second dog received a total of 1,942 milligrams per kilogram body weight in 20 days, or 97 milligrams per kilogram body weight per day, which amounts to a total of 1,359 grains per 100 pounds body weight, or 68 grains per 100 pounds body weight per day. In both cases a distinct amount of albumen was present in the urine, and the urine evolved sulphurous acid on treatment with hydrochloric acid. The third dog received hypodermically three doses in eight days, each dose being 213 milligrams per kilogram body weight; that is, 149 grains per 100 pounds body weight; no bad effects. (*p. 133.*)
3. "Administered by the stomach Azarin S is harmless." (*p. 134.*)
4. "Other Azo colors, * * * for instance Azarin S, are entirely nonpoisonous." (*p. 148.*)
5. **Fraenkel** (*p. 578*): "Azarin S administered by the stomach is entirely harmless."

UNFAVORABLE.

1. **Weyl** (*p. 134*): Dog; weight not given; received 5 cc of Azarin S paste by injection into the abdominal cavity, and survived three days. "The cause of death was considered to be peritonitis without effusion. The result of this post-mortem is of much interest. The red spots consisted, as was determined by chemical analysis, of the azo color which is the basis of the Azarin S. Consequently in the peritoneal cavity the same splitting up of the Azarin S had occurred which takes place when it is attached to textiles."
2. **Fraenkel** (*p. 578*): To the same effect.

G. T. 78.

Trade name.—Erika B.

Scientific name.—Sodium salt of methyl-benzenyl-amido-thio-xylenol-azo-alpha-naphthol-disulphonic acid.

Discovered and patented.—1889.

Shade.—Rose Pink. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 153*): Based on his own experiments considers it as not harmless. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
May 9	2	8.0	392	Dog normal; urine acid; no albumen.
10			400	Urine of rose shade; insignificant traces of albumen; acid.
11			420	Color same; no albumen.
12	2		360	Do.
13			390	Do.
14			293	Urine yellow, greenish shade; traces of albumen.
15			350	Color normal; no albumen.
16	2	8.2	390	Do.
17			402	Urine wine yellow, orange, acid; no albumen.
18			350	Dog quite well; urine orange; no albumen.
Total.	6			

G. T. 84.

Trade names.—Resorcin Yellow; Tropæolin O; Tropæolin R; Chrysoin; Chryseolin; Yellow T; Gold Yellow; Acme Yellow.

Names under which it was offered on the United States market as a food color in 1907.—Chrysoin REZ; Resorcin 0275.

Scientific name.—Sodium salt of para-sulphobenzene-azo-resorcinol.

Discovered.—1875.

Shade.—Reddish yellow. Offered by 2 out of 12 sources.

FAVORABLE.

1. Chlopin (*pp. 131-2*) examined this color physiologically, and has classified it as harmless. The experimental data are as follows:

Experimental data by Chlopin.

No. 1 (*p. 227*).

[1 gram=43 mg=30 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 9		23.00	500	Dog quite well; urine normal color, acid; no albumen.
10	[1] 1.21			Do.
11	[2] 0.70			Urine acid; no albumen.
12	[1] 2.00			No symptoms of poisoning; eats.
13			460	Urine dark brown; no albumen.
14			520	Do.
17			580	Urine normal color; no albumen; dog is well.
Total..	[1] 3.21 [2] 0.70			

No. 2.

[1 gram=156 mg=109 grains.]

1901.				
Oct. 9		6.4	30	Dog well; urine yellow; no albumen.
10	2		350	Do.
11	2			Urine brown, acid; no albumen.
12			300	Urine light brown, acid; no albumen.
13	2		335	Do.
14			290	Do.
15			290	Do.
16	2		375	Do.
17			300	Do.
18	2			Do.
19			320	Urine light brown, acid, no albumen; diarrhea.
20			360	Brown yellow, acid, no albumen no diarrhea.
21			360	Urine normal; dog is well.
22-26		7.0		Do.
Total...	10			

[1] Internally. [2] Subcutaneously.

2. Permitted by the law of Italy.

UNFAVORABLE.

1. Forbidden by the Confectioners' List.

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; not indifferent.

G. T. 85.

Trade names.—Orange I; Alphanaphthol Orange; Naphthol Orange; Tropæolin OOO; Orange B.

Names under which it was offered on the United States market as a food color in 1907.—Orange RZ; Orange 027.

Scientific name.—Sodium salt of para-sulphobenzene-azo-alpha-naphthol.

Discovered.—1876.

Shade.—Orange. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): "According to Cazeneuve and Lépine's experiments, the following are not poisonous to human beings and dogs: * * * Orange * * *."
3. "Orange I (Alpha-naphthol Orange, Tropæolin OOO) not poisonous to dogs neither by administration by stomach, nor by injection into blood." (On authority of Cazeneuve and Lépine.) (*p. 115.*)
4. **Weyl** (*pp. 123, 148*) refers to this as not poisonous.
5. Permitted by the law of Italy.
6. Permitted by the law of Austria.
7. **Cazeneuve** (*Arch. gén. de méd., 1886, Vol. I, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
8. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, well or sick.

G. T. 86.

Trade names.—Orange II; Betanaphthol Orange; Tropæolin OOO No. 2; Mandarin G extra; Chrysaurein; Gold Orange; Orange extra; Atlas Orange; Orange A.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Yellow SLOZ; Orange II; Orange; Orange Y; Mandarin G extra; Orange A 1201; Orange A extra.

Scientific name.—Sodium salt of para-sulphobenzene-azo-beta-naphthol.

Discovered.—1876.

Shade.—Orange. Offered by 8 out of 12 sources.

FAVORABLE.

1. Permitted by law in Italy.
2. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, p. 974*) says that according to his experiments this color, in the small amounts in which it is used in food products and which can enter the human system in the course of 24 hours, can hardly, even with frequent administration, cause a harmful effect.
3. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, pp. 968–974*): Experimented on rabbits, giving dye with food, a total of 21 grams in 19 days, a total of 8,748 milligrams per kilogram body weight, or 6,133 grains per 100 pounds body weight, in doses of 1 gram each, daily for the first 15 days, or about 417 milligrams per kilogram of body weight, or 292 grains per 100 pounds body weight per dose for these 15 doses; the color could only be detected in the urine, and the feces became softer. A dog was given 1,020 milligrams per kilogram body weight, or 714 grains per 100 pounds body weight, and showed distinct kidney irritation, great thirst, and diarrhea; recovery required about one week; and thereafter the same animal was fed by the mouth one-twentieth of the above dose each day for 9 successive days without any untoward effect. A second dog received per kilogram of body weight 172 milligrams, or 121 grains per 100 pounds body weight, and it, like the first dog, in the first experiment, showed kidney irritation, diarrhea, and great thirst. On humans 100 milligrams, or 1½ grains, colored the urine within 15 minutes, and this color remained for 24 hours; there was no sign of vomiting or diarrhea; the bitter taste of the color was noticeable.

UNFAVORABLE.

1. Forbidden by Confectioners' List.
2. Forbidden by Swiss Analytical chemists.
3. Weyl (*p. 115*): "Poisonous Orange II. * * *."
4. "Betanaphthol Orange is, therefore, according to Experiment I, poisonous in small doses when administered by the stomach, and suffices to kill an ordinarily large strong dog." (*p. 123*)
5. A. Weyl's own experiments on two dogs, the initial dose in one case being 476 milligrams per kilogram body weight, or 333 grains per 100 pounds body weight; the animal receiving in the course of 20 days, in four doses, 1,333 milligrams per kilogram body weight, or 933 grains per 100 pounds body weight; or 335 milligrams per dose per kilogram body weight; that is, 225 grains per dose per 100 pounds body weight. The animal died, and suffered diarrhea and albuminuria, and its urine was colored red throughout the entire period. B. The second dog received hypodermically per kilogram body weight, 116 milligrams, or 61 grains per 100 pounds body weight; its urine was colored; albuminuria, diarrhea, loss of hair, abscesses, and loss of weight occurred. It required 36 days to recover from four doses administered during one week. C. A rabbit received 1,333 milligrams per kilogram body weight, or 933 grains per 100 pounds body weight, and died within 12 hours. (*p. 122*)
6. "Of the 23 Azo colors subjected to examination only two * * * Orange II produce(s) such effects when administered by the stomach that we can consider it poisonous. With dogs the lethal dose is less than 1 gram per kilo of the body weight of Orange II * * *." (*p. 147.*)
7. "Further Orange II, which is poisonous * * *."
8. "The poisonous qualities of Orange II."
9. "Further, in spite of the presence of the sulpho groups, colors may be poisonous, as is shown with Orange II." (*p. 148.*)
10. Chlopin (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*): A. A dog received 349 milligrams per kilogram body weight, or 244 grains per 100 pounds body weight for the first day of experiment, which dose was repeated on the third and fourth days; nothing untoward is noted for the first three days in the condition of the dog; the urine was dark red but free from albumen; on the fifth and sixth days two-thirds of the above amount was given, and on the seventh and eighth days the original dose was given. On the fourth day the animal was frisky and had a good appetite but was vomiting; on the fifth day vomiting stopped, but diarrhea ensued, which diarrhea continued for one week; the weight remained practically constant; the urine was colored throughout from dark red to orange red and dark brown and became normal the fourth day after the last administration. B. Humans: Chlopin took 200 milligrams, or $3\frac{1}{10}$ grains, in a gelatin capsule at 3 p. m.; at 4.30 p. m. the urine was colored a strong red orange; at 6 p. m. a dryness of throat and bad taste in mouth appeared; at 6.30 p. m. felt very badly; vertigo and unable to remain seated and continue writing; blood rushed to head; the general condition very poor; somewhat improved by moving about in open air; 7.30 felt so poorly took Glauber's salt as an antidote; 11 p. m. ill condition still continuing; urine normal yellow; midnight recovered.

    Chlopin states that he would not repeat this experiment on himself, or on any other human, and he concludes therefore that this color must be regarded as harmful.
11. Chlopin (*p. 133*) classes it as "harmful." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=116 mg=81 grains.]

Date.		Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.		*Grams.*	*Kilos.*	*cc.*	
May	27	3	8.6	350	Dog is quite well; urine normal.
	28			340	Urine brown red, acid; no albumen.
	29	3		350	Do.
	30	3		350	Diarrhea and vomiting at night, no albumen; urine orange red, acid, no albumen.
	31	2		325	Diarrhea continues; no albumen; no vomiting.
June	1	2		330	Diarrhea continues; dog lively, eats with relish; no albumen.
	2	3		350	Urine brown orange, acid, no albumen; diarrhea continues.
	3	3	8.2	315	Urine clear; no albumen; diarrhea continues.
	4			375	Urine dark brown; no albumen; diarrhea decreases.
	5				Diarrhea still less; no albumen.
	6		8.59		Diarrhea very slight; urine slightly orange, no albumen.
	8			350	Urine normal in color and composition; diarrhea stopped.
Total..		19			

12. Buss lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 87.

Trade names.—Orange III; Helianthin; Tropæolin D; Methyl Orange; Dimethylanilin Orange.

Scientific name.—Sodium salt of para-sulphobenzene-azo-dimethylanilin.

Discovered.—1876.

Shade.—Orange Yellow. Not offered.

FAVORABLE.

1. Permitted by the law of Italy.

UNFAVORABLE.

1. **Chlopin** (*pp. 145, 146*) on his own experiments classes it as poisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=163 mg=114 grains.]

Date.		Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.		*Grams.*	*Kilos.*	*cc.*	
Aug.	29		6.14	310	Before experiment dog quite normal; color urine usual, acid, no albumen.
	30	2		250	Do.
	31			330	Color of urine dark yellow; no albumen.
Sept.	1	2		305	Urine brown; reddish with sulphuric acid; no albumen.
	2			305	Do.
	3				Do.
	4	2			Do.
	5	2			Do.
	6	2		Little.	Urine dark brown; no albumen; vomiting.
	7			Little.	Paresis of hind legs; dog does not eat, but drinks with avidity.
	8–9			Little.	Complete ataxia, which became general on the 9th; dog can not move in straight line, and walks in circles, and drops his head on things and falls; after falling arises with difficulty and stands with widely spread feet, continuously lifting now one then the other; extremities shake; on 11th day sat with difficulty; placed on his feet, maintained equilibrium with difficulty; involuntarily lifts one paw and moves it about in the air; animal can still swallow; eyes respond to light; does not eat; on the 12th the hind legs completely paralyzed; on the 14th vomiting and paralysis of the front legs; lies quietly without moving; is killed. Autopsy shows no change in internal organs except hyperæmia in the lumbar region of the "spinal column (?) bordering between anterior and lateral columns," causing death by paralysis of the heart.
Total..		10			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram=149 mg=104.3 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 3	3	6.7	....	A few hours after giving color dog in tremors; does not eat.
4	....	....	Little.	Urine black; acid; no albumen; paralysis of the extremities.
5	....	....	Little.	Dog lies in cage in full paralysis; died before dinner; no albumen in urine; cause of death, paralysis of heart.

2. **Meyer** (*J. Amer. Chem. Soc., 1907, v. 29, p. 900*): Dog receiving 113 milligrams per kilogram body weight; that is, 79 grains per 100 pounds body weight. Result, diarrhea, which continued throughout 17 days, although only 3 doses of the same size were given in 6 days; thereafter the dose was increased geometrically; the diarrhea continued; the coloring matter was discharged in the urine and the feces; there was no abnormal condition revealed by the autopsy.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 88.

Trade names.—Diphenylamin Orange; Orange IV; Tropæolin OO; Orange M; Fast Yellow; Orange G S; New Yellow; Orange N; Acid Yellow D.

Scientific name.—Sodium salt of para-sulphobenzene-azo-diphenylamin.

Discovered.—1876.

Shade.—Orange Yellow. Not offered.

FAVORABLE.

1. **Weyl** (*p. 115*): "Nonpoisonous * * * Diphenylamin Orange * * *
2. * * * Diphenylamin Orange is * * * nonpoisonous." (*p. 132.*)
3. "For instance, the poisonous Metanil Yellow corresponds to the nonpoisonous Diphenylamin Orange." (*p. 148.*)
4. The experiments on which Weyl based the conclusions above may be summarized as follows: A. A dog received 183 milligrams per kilogram body weight, or 128 grains per 100 pounds body weight. The urine was rendered black, and contained traces of phenol and abundant albumen; this condition lasted for 72 hours, at the end of which 110 milligrams per kilogram body weight, or 77 grains per 100 pounds body weight, were administered; urine became colorless and albumen diminished. Five days afterwards a total of 10 grams, or 366 milligrams per kilogram body weight, or 256 grains per 100 pounds body weight, were administered; the urine became abundant, was strongly black, alkaline, contained albumen. The loss of weight was 1 kilogram, or about 3½ per cent. B. A dog received 308 milligrams per kilogram, or 216 grains per 100 pounds body weight, as the initial dose; albuminuria did not result until after repeated dosing same as initial dose, and administered for 2 weeks.
5. Weyl's conclusions are as follows: "According to the above investigations, Diphenylamin Orange causes albuminuria, but further disturbances did not appear during the several weeks' observations on the animals used."

6. **Chlopin** (*p. 148*) examined this color and classes it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram—145 mg—100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Oct. 27	3	7	300	Dog quite normal; urine color normal; no albumen.
28				Urine dark brown; acid; no albumen.
29	3			Do.
30	3			Do.
31	3			Do.
Nov. 1	3			Urine yellow, with sulphuric acid red; no albumen.
2	3		340	Orange with orange sheen; no albumen.
3	3	6.5		Do.
4			275	Urine brown yellow; acid; no albumen.
5	3		300	Do.
6		6.7		Do.
Total.	24			

7. **Fraenkel** (*pp. 577, 578*) to the same effect as Weyl, as above.
8. Permitted by the law of Italy.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 89.

Trade names.—Brilliant Yellow S; Yellow WR; Curcumin.

Names under which it was offered on the United States market as a food color in 1907.—Brilliant Yellow S.

Scientific name.—Sodium salt of para-sulphobenzene-azo-diphenylamin-suphonic acid.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Lieber** (*p. 136*): A very young rabbit received six doses on alternate days, each dose amounting to 320 milligrams per kilogram of body weight, or 224 grains per 100 pounds of body weight. No untoward symptoms are recorded; the body weight increased almost 10 per cent in 11 days.

UNFAVORABLE.

Nothing.

G. T. 92.

Trade names.—Azo Acid Yellow; Azoflavin; Azo Yellow; Indian Yellow.

Scientific name.—Mixture of nitrated diphenylamin yellow with nitro-diphenylamin.

Discovered.—1880.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Chlopin** (*p. 128*) examined this color and classifies it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 6	2	8	370	Dog normal; urine acid; no albumen.
8			Little.	Urine reddish orange; acid; no albumen.
10	3			Do.
11			600	Urine red; no albumen.
12			430	Urine almost normal color; acid; no albumen.
Total.	5			

Conclusion: Nonpoisonous.

No. 2 (*p. 129*).

[1 gram=143 mg=100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 12	2	7	430	Dog normal; urine acid; no albumen.
14	3		610	Do.
15			600	Urine dark yellow; acid; no albumen.
16			400	Urine almost normal color; acid; no albumen.
Total.	5			

Conclusion: Showed no harmful effects.

2. Permitted by law in Italy.

UNFAVORABLE.

Nothing.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion; noticeably retards digestive action; is not indifferent.

G. T. 93.

Trade name.—Azo-fuchsin G.

Scientific name.—Sodium salt of para-sulphobenzene-azo-dioxy-naphthalene-sulphonic acid.

Discovered.—1889.

Shade.—Reddish brown. Not offered.

FAVORABLE.

1. **Chlopin** (*pp. 126, 127*) examined this color and classifies it as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=117 mg=82 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901	*Grams.*	*Kilos.*	*cc.*	
May 3	2	8.5	300	Dog well and normal; urine acid; no albumen.
4			363	Urine chocolate brown; acid; no albumen.
5	2		390	Do.
6			380	Urine dark brown, chocolate, in thin layer greenish sheen; acid; no albumen.
7	2		285	Urine slightly greenish; acid; no albumen.
8			420	Dark chocolate brown; acid; no albumen.
9		8.7	292	Urine normal; dog is well.
Total.	6			

No. 2.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.				
Oct. 9		8.0	390	Dog quite normal; urine acid; no albumen.
10	2		310	Do.
11	2			Urine slightly more yellow; no albumen.
12			380	Urine dark brown; acid; no albumen.
13	2		325	Do.
14				Do.
15	2		396	Do.
16	2			Do.
17			397	Do.
18	2		325	Do.
19			380	Urine almost black; acid; no albumen.
20			246	Urine almost black; acid; no albumen; urine of lighter color.
21		8.4	420	Do.
Total.	12			

UNFAVORABLE.

Nothing.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 94.

Trade names.—Tartrazin; Hydrazin Yellow.

Names under which it was offered on the United States market as a food color in 1907.—Flavazein Red Shade Z; Tartrazin; Acid Yellow AT.

Scientific name.—Sodium salt of benzene-azo-pyrazalone-carboxy-disulphonic acid.

Discovered.—1884.

Shade.—Yellow. Offered by 6 out of 12 sources.

DOUBTFUL.

1. **Lieber** (*p. 134*): Dog, 2 months old, received, per kilo, body weight, 37 milligrams, or 26 grains per 100 pounds body weight, six times, on alternate days. The animal suffered from mild diarrhea at the start, which continued with greater or less activity than at the start throughout the test.

2. **Meyer** (*J. Amer. Chem. Soc., 1907, v. 29, p. 897*): Dog received 100 milligrams per kilogram body weight, or 70 grains per 100 pounds, increased in geometric proportion for 6 consecutive days, at the end of which time diarrhea set in. On that day 2,000 milligrams per kilogram body weight, or 1,400 grains per 100 pounds body weight, were administered; this was about two-thirds as much as the animal had received in all the 5 days preceding; the feçes were colored after the first administration, and the urine was also colored; albuminuria doubtful.
3. **Fraenkel** (*pp. 210 and 216*): Tartrazin, according to the Green Tables, is a derivative of isopyrazolon; "* * * only those substances which are derivatives of pyrazolon are antipyretics, the isopyrazolon derivatives are, however, poisonous." Tartrazin is also closely related to the antipyrin class of compounds, which class is known to possess an irritant action and also a depressing action on the circulation. Tartrazin also contains benzol groups, which are said to increase the physiological activity of pyrazolon derivatives.

G. T. 95.

Trade names.—Metanil Yellow; Orange MN; Tropæolin G.

Names under which it was offered on the United States market as a food color in 1907.—Victoria Yellow conc. Z; Yellow MXX conc.

Scientific name.—Sodium salt of meta-sulphobenzene-azo diphenylamin.

Discovered.—1879.

Shade.—Orange. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Frentzel** (*Zts. Nahr. Genussm., 1901, v. 4, p. 974*): A. A rabbit received 379 milligrams per kilogram body weight, or 265 grains per 100 pounds body weight; no color administered for 2 days, and then administered at intermittent periods, so as to receive 6 doses in 19 days. Total weight administered per kilogram body weight 2,085 milligrams, or 1,460 grains per 100 pounds, which is equivalent to 77 grains per day per 100 pounds; the color could always be detected in the urine, and the feces became softer. B. A dog received 581 milligrams per kilogram body weight at one dose; that is, 407 grains per 100 pounds body weight. There was no vomiting and no diarrhea; the dye persisted in the urine for 72 hours. C. The same dog, after recovering from the foregoing, received daily one-tenth the above dose for 9 days; the dyestuff was found in the urine and the feces and movements were normal; dissection showed nothing abnormal. D. A human swallowed 100 milligrams, or 1.5 grains; no untoward symptoms are recorded; the urine remained colored for 24 hours. From the foregoing Frentzel concludes that this coloring matter is absolutely harmless.
2. **Chlopin** (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*): A. A dog received 305 milligrams per kilogram body weight; that is, 104 grains per 100 pounds body weight; no color administered for four days; the same dose was given on alternate days six times, and the dose was increased 50 per cent, and that dose administered twice, and the original dose was given on the next alternate day; altogether the animal received per kilogram body weight, 3,355 milligrams, or 1,144 grains per 100 pounds body weight, in a period of 22 days, or an average of 153 milligrams per kilogram body weight per day, or 52 grains per day per 100 pounds body weight. At the end of the twelfth day albuminuria set in, and it required three weeks after ceasing the administration of the color for the albuminuria

2. **Chlopin** (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*)—Continued.
to disappear; the urine was colored throughout the period of dosing, and it was not until 10 days after the last color had been administered that the urine regained normal color; otherwise the animal was well throughout. B. Humans: Two hundred milligrams, or 3$\frac{1}{11}$ grains taken at 3 p. m., October 30, 1901, colored the urine so highly yellow that it could be dyed with. Apart from the bitter taste of the product no untoward symptoms are recorded. C. Chlopin's conclusion (*Zts. Nahr. Genussm., 1902, v. 5, p. 244*): "Metanil Yellow is not poisonous to dogs in doses of from 2 to 3 grams per day, nor to humans in doses of 0.2 grams per day, and may therefore perhaps be regarded as nonpoisonous from a practical point of view."

UNFAVORABLE.

1. **Weyl** (*p. 115*): "Poisonous * * * Metanil Yellow."
2. Says the product smelled strongly of diphenylamin. (*p. 130.*)
3. A. "Metanil Yellow must be considered poisonous when administered by the stomach from the indications of Experiments 1 and 2. The lethal dose, which is determined by Experiment 2, is 0.53 grams per kilo body weight." This lethal dose is 371 grains per 100 pounds body weight. B. A dog received 862 milligrams per kilogram body weight, or 603 grains per 100 pounds body weight. This caused vomiting; the same dose was repeated 24 hours afterwards, the animal again vomiting. The animal died within 96 hours from the first administration. C. A dog received 89 milligrams per kilogram body weight, or 62 grains per 100 pounds body weight; the urine became colored about 96 hours after administration; 5 days after the first administration the animal was given 10 times the original dose; vomiting set in within one hour; in 24 hours the urine was deeper colored; a week later after the dose last preceding, one-half of that dose was given, and the animal died within 24 hours; the animal had lost during this period approximately one-quarter its original weight. (*p. 132.*)
4. "* * * Metanil Yellow * * * produce[s] such effects when administered by the stomach that we can consider them [it] poisonous." (*p. 147.*)
5. "The poisonous qualities of * * * Metanil Yellow; the poisonous Metanil Yellow." (*p. 148.*)
6. **Fraenkel** (*p. 578*): "A dog weighing 11 kilograms was killed by 20 grams of this coloring matter within four days, whereas the isomeric Diphenylamin Orange is nonpoisonous, and it must first be considered if the poisonous nature of this substance can be explained by the easy liberation of diphenylamin from it, since this coloring matter, in and of itself, has a strong odor of diphenylamin."
7. **Chlopin** (*p. 141*): Based on his own experiments considers it "Not quite harmless." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram = 153 mg = 107 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
May 18		6.55	390	Quite normal.
19	2		350	Urine unusual color; acid; no albumen.
20			308	Do.
21			300	Urine orange; acid; no albumen.
22	2		370	Do.
23				Do.
24	2		380	Do.
25			332	Urine dark brown, no albumen.
26	2		320	Do.
27			365	Do.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 28	2	....	330	Urine dark brown, no albumen.
29	....	....	350	Do.
30	2	....	350	Do.
31	....	....	355	Do.
June 1	2	....	400	Urine brown; traces of albumen.
2	....	....	340	Do.
3	....	....	330	Do.
4	3	....	400	Do.
5	....	....	305	Do.
6	3	....	390	Do.
7	....	....	330	Do.
8	2	....	335	Do.
9-29	....	6.5	....	Color of urine normal June 18, on 29th albumen disappears. Dog is well.
Total..	22			

8. **Chlopin** (*p. 141*): On authority of others not stated, classes this color as harmful or poisonous.
9. Prohibited by the laws of Italy.
10. Prohibited by Confectioners' List.
11. Prohibited by the Resolutions of the Society of Swiss Analytical Chemists, September, 1891.
12. Buss lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says that it noticeably retards digestion.

G. T. 97.

Trade names.—Orange T; Mandarin G R; Orange R; Kermesin Orange.

Name under which it was offered on the United States market as a food color in 1907.—Orange 2 R.

Scientific name.—Sodium salt of sulpho-ortho-toluene-azo-beta-naphthol.

Shade.—Orange. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 125*): Based on his own experiments considers this color as "harmful." The experimental data are as follows:

No. 1.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 4	1	....	310	Nothing abnormal.
5	....	....	280	Urine reddish brown; traces of albumen; acid.
6	3	....	420	Do.
7	....	....	570	Urine dark brown; acid; traces of albumen.
8	....	....	370	Color of urine weaker; no albumen.
9	....	....	250	Almost normal-colored urine; no albumen; dog is well and lively.
10	....	....	330	Nothing abnormal.
Total..	4			

No. 2.

[1 gram = 217 mg = 152 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 25	2	4.6	200	Soon after giving color heavy vomiting and diarrhea; dog does not eat or drink the rest of the day; urine acid; no albumen.
26	....	....	....	Vomiting and diarrhea continued; dog is tired and run down, and began to eat; urine dark brown and no albumen.
27	....	....	....	Urine same; dog livelier.
28	2	4.3	180	Diarrhea and vomiting stopped; urine of brown color; acid; no albumen.
29	2	....	200	Urine orange; no albumen; acid.
Total..	6			

G. T. 102.

Trade names.—Fast Red; Roccellin; Cerasin; Rubidin; Fast Red A; Rauracienne; Orcellin No. 4.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol.

Shade.—Brownish red. Not offered.

Discovered and patented.—1877.

FAVORABLE.

1. Permitted by the law of Italy.
2. Permitted by the law of Austria.
3. Cazeneuve (*Arch. gen. de méd., 1886, p. 753*) says it may be taken without effect by man or animals, sick or well, in large doses.
4. Cazeneuve and Lépine (*Bull. de l'acad. de méd. 1886, p. 643*): Tolerated by man well or sick.

G. T. 103.

Trade names.—Azorubin S; Carmoisin; Azo Acid Rubin; Fast Red C; Azorubin A.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Cardinal 3 B; Azorubin; Carmoisin B.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-alpha-naphthol-para-sulphonic acid.

Discovered and patented.—1883.

Shade.—Red. Offered by 6 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Cazeneuve and Lépine: Not poisonous to human beings.
3. Meyer (*J. Amer. Chem. Soc. 1907, v. 29, p. 898*): One hundred milligrams per kilogram body weight, or 70 grains per 100 pounds body weight administered, increased geometrically; diarrhea was marked only after administration of exceptionally large doses (the seventh day); the stools were deep violet and the urine was carmine, becoming dark; the autopsy developed nothing abnormal; the whole interior was of a red color.

4. **Lieber** (*p. 138*): A guinea pig received once a day six times every other day 241 milligrams per kilogram body weight, or 169 grains per 100 pounds body weight; appetite remained good throughout, and aside from an occasional thirstiness noted no untoward observations were recorded.

G. T. 105.

Trade names.—Fast Red E; Fast Red.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-monosulphonic acid.

Discovered and patented.—1878.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Meyer** (*J. Amer. Chem. Soc. 1907, v. 29, p. 898*): Initial dose 100 milligrams per kilogram body weight or 70 grains per 100 pounds, and given three times increased geometrically once before diarrhea was observed; there was no albumen in the urine; urine was colored deep red; feces colored red at the start, at the end of the experiment chocolate brown; autopsy showed all parts to be substantially normal.
3. **Cazeneuve and Lépine** (*Compt. rend., 1885, v. 101, pp. 823–827*): A. Dog: Weight 21.5 kilo, received as follows:

Days.	Grams.	Milligrams per kilo.	Grains per 100 pounds.
32	0.500	23.2	16.2
20	2.150	100.0	70.0
8	4.300	200.0	140.0
10	5.000	232.0	162.0
5	10.000	462.0	324.0

Nothing abnormal except occasional greenish urine; no vomiting; no diarrhea. B. Man: 1. One of the experimenters took 1 gram, dissolved in wine, daily for 15 days; no effect. 2. A man aged 25, afflicted with albuminuria, received the following:

Days.	Grams.
3	0.5
2	1.0
1	2.0

Caused colic without diarrhea; amount of urine or albumen not affected. 3. Three men afflicted with Bright's disease received each daily for 8 days 1 gram; no effect on the albumen. 4. A 30-year-old man chronic invalid took 4 grams one day, 6 grams the next day; no effect was observed.

4. **Arloing and Cazeneuve** (*Archives de physiologie, 1887, pp. 356–393*): As the result of this work, which is divided into four parts: (1) Stating the effect of direct introduction of the color into the circulation; (2) intravenous injections; (3) comparing the effects of injections of color and of salt; and (4) feeding by the mouth—these investigators conclude that these coloring matters are toxic only in extremely large doses; that when given to dogs with their food no inconvenience of any kind results; this is based upon experiments upon three dogs, covering 145 days, where each dog received, per kilogram initial body weight, in the first case, 20,307 milligrams, or 14,215 grains per 100 pounds initial body weight; in the second case, 29,590 milligrams, or 20,713 grains per 100 pounds initial body weight; in the third case, 28,154 milligrams, or 19,758 grains per 100 pounds initial body weight. Per day this would mean 98 grains per 100 pounds initial body weight in the first case; in the second case the daily dose was 143 grains per 100 pounds initial body weight; and in the third case the daily dose was 137 grains per 100 pounds initial body weight.

G. T. 106.

Trade names.—New Coccin; Cochineal Red A; Brilliant Scarlet; Crocein Scarlet 4 BX.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Crocein Scarlet; New Coccin; Scarlet L; Brilliant Scarlet 4 R.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-disulphonic acid (G.).

Discovered.—1878. Offered by 5 out of 12 sources.

Shade.—Red.

FAVORABLE.

1. **Cazeneuve and Lépine.** (See *Weyl, p. 115.*)
2. **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs, * * * Purple * * *"
3. **Cazeneuve and Lépine** (*Bull. de l'acad. de méd., 1886, p. 643*): Tolerated by man, sick or well.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 107.

Trade names.—Fast Red D; Azo Acid Rubin 2 B; Fast Red E B; Bordeaux S; Amaranth.

Names under which it was offered on the United States market as a food color in 1907.—Claret Red RZ; Red; Amaranth B (Azo color similar to); Bordeaux S; Naphthol Red S; Amaranth.

Scientific name.—Sodium salt of para-sulphonaphthalene-azo-beta-naphthol-disulphonic acid (R).

Discovered and patented.—1878.

Shade.—Red. Offered by 7 out of 12 sources.

FAVORABLE.

1. **Cazeneuve and Lépine** (see *Weyl, p. 115*): Not poisonous to human beings.
3. **Lieber** (*p. 148*): A rabbit received 284 milligrams per kilogram body weight, or 199 grains per 100 pounds once a-day, five times every other day, and aside from decreased appetite the second and third day of the observation period nothing untoward is noted.
4 **Weyl** (*p. 31*): "* * * not poisonous to human beings and dogs, * * * Purple * * *"
5. **Cazeneuve and Lépine** (*Bull de l'acad. de méd., 1886, p. 643*): Tolerated by man, sick or well.

G. T. 138.

Trade names.—Fast Brown G; Acid Brown.

Scientific name.—Sodium salt of bi-sulphobenzene-disazo-alpha-naphthol.

Discovered.—1882.

Shade.—Brown. Not offered.

FAVORABLE.

1. **Weyl** (*p. 134*): "The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz, Fast Brown G * * *. All these proved to be nonpoisonous * * *." A. A dog weighing 9.63 kilos received 312 milligrams per kilogram body weight, or 218 grains per 100 pounds body weight; the same dose was repeated 48 hours afterwards, when diarrhea set in, and the urine was colored red; 24 hours afterwards 208 milligrams per kilogram body weight, or 146 grains per 100 pounds body weight were given; the urine was colored strongly red 24 hours thereafter. Eight days afterwards 520 milligrams per kilogram body weight, or 364 grains per 100 pounds body weight, were administered; marked diarrhea set in; 3 days later the dose given was twice the dose last given, when severe diarrhea resulted, but unchanged color appeared in the urine, and continued for 24 hours afterwards; diarrhea continued for 96 hours. Evidences of albuminuria apparently not dependable. B. A second dog, weighing 5.9 kilos, received 339 milligrams per kilogram body weight, or 237 grains per 100 pounds body weight daily throughout an entire month; diarrhea was produced after 6 days, which continued almost during the month; appetite was diminished and the loss in weight was about 20 per cent on the original. C. Weyl concludes as follows: "According to these experiments, this color in continuous, though slight, doses, or in large doses, but less frequently, produces diarrhea, anorexia, and emaciation." (*p. 136.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 147*): "Of the remaining colors * * * others (produce) diarrhea (Fast Brown * * *)."

G. T. 160.

Trade name.—Crocein Scarlet 3 B; Ponceau 4 RB.

Scientific name.—Sodium salt of sulphobenzene-azo-benzene-azo-beta-naphthol-monosulphonic acid.

Discovered and patented.—1881.

Shade.—Scarlet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

DOUBTFUL.

1. Houghton (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–57*): Hinders fibrin digestion at all strengths; at 1:200 hinders casein and albumen digestion.

G. T. 163.

Trade names.—Biebrich Scarlet; Ponceau B; New Red L; Ponceau 3 RB; Fast Ponceau B; Imperial Scarlet.

Scientific name.—Sodium salt of sulphobenzene-azo-sulpho-benzene-azo-beta-naphthol.

Discovered.—1878.

Shade.—Scarlet. Not offered.

FAVORABLE.

1. Permitted by law in Italy. (See *Lieber, pp. 18, 22, 23.*)

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 164.

Trade name.—Crocein Scarlet O extra.

Scientific name.—Sodium salt of sulphobenzene-azo-sulphobenzene-azo-beta-naphthol-sulphonic acid.

Discovered.—1888.

Shade.—Scarlet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Prohibited by Confectioners' List.

G. T. 166.

Trade name.—Wool Black.

Scientific name.—Sodium salt of sulphobenzene-azo-sulphobenzene-azo-para-tolyl-beta-naphthylamin.

Discovered.—1885.

Shade.—Black. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 134*): "The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz: * * * Wool Black * * *. All these proved to be nonpoisonous * * *."

3. A. A dog received 167 milligrams per kilogram body weight, or 117 grains per 100 pounds body weight each day for 3 successive days; no color was administered for the next 2 days, and the third day the dose was double the former dose. The urine was colored bluish-black and contained albumen. Two days afterward the same dose was given; urine of intense dark-blue color, and contained unaltered coloring matter, which disappeared in 48 hours. Albuminuria continued for about 15 days. (*p. 137.*) B. "Wool Black is nonpoisonous both by gastric and by subcutaneous administration." (*p. 137.*)

G. T. 169.

Trade names.—Crocein Scarlet 7 B; Ponceau 6 RB; Crocein Scarlet 8 B.

Names under which it was offered on the United States market as a food-color in 1907.—Sodium salt of sulphotoluene-azo-toluene-azo-beta-naphthol-alpha-sulphonic acid.

Discovered and patented.—1881.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 188.

Trade names.—Naphthol Black B; Brilliant Black B.

Name under which it was offered on the United States market as a food-color in 1907.—Naphthol Black BDF.

Scientific name.—Sodium salt of disulpho-beta-naphthalene-azo-alpha-naphthalene-azo-beta-naphthol-disulphonic acid.

Discovered and patented.—1885.

Shade.—Black. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 138*): A. A dog received 112 milligrams per kilogram body weight, or 78 grains per 100 pounds body weight, daily for 3 successive days; distinct albuminuria, uncolored urine; blue-colored feces; thereupon the daily dose was increased to 187 milligrams per kilogram body weight, that is, 131 grains per 100 pounds. No color was administered for 6 days, and during this time the urine was colored from reddish-violet to a bluish-black red; thereupon the last dose was doubled and 24 hours afterwards that dose was doubled; rather much albumen in urine which was bluish; albuminuria continued for about a week. B. A dog received 222 milligrams per kilogram body weight, or 155 grains per 100 pounds daily throughout a whole month; it remained entirely well with good appetite.
3. "This color is harmless when administered by the stomach, but poisonous subcutaneously." (*p. 140.*)
4. Schacherl: Not harmful under conditions in which it is used.

UNFAVORABLE.

1 Weyl (*p. 139*): Where a dog, receiving subcutaneously 31 milligrams per kilogram body weight, or 22 grains per 100 pounds body weight, subcutaneously, died apparently wholly as a result of the color.

2. "Naphthol Black P, however, is plainly poisonous when introduced into the subcutaneous cellular tissue." (*p. 147.*)

G. T. 197.

Trade names.—Bismarck Brown; Phenylene Brown; Leather Brown; English Brown; Manchester Brown; Vesuvin; Cinnamon Brown.

Names under which it was offered on the United States market as a food-color in 1907.—Vesuvin 4B Conc. Z; Bismarck Brown; Bismarck Brown B No. 216; Bismarck Brown B.

Scientific name.—Hydrochlorid of benzene-disazo-phenylene-diamin,

Discovered and patented.—1863.

Shade.—Reddish brown. Offered by 4 out of 12 sources.

FAVORABLE.

1. **Weyl** (*p. 115*): "Nonpoisonous Bismarck Brown."

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 117*): A. A dog received 33 milligrams per kilogram body weight, or 23 grains per hundred pounds body weight; in one and one-half hours, vomiting; next day same dose same result with the addition that the animal took no food and moved about but little for 48 hours; on the fourth day same dose, in two hours, vomiting; for 96 hours animal took no food; on the eighth day albumen in the urine and the animal ate; on the ninth day 83 milligrams per kilogram body weight or 58 grains per 100 pounds body weight, vomited for one-half hour after administration; for the next 5 days the animal took hardly any food; on the fourteenth day the animal improved, took food on the fifteenth day; traces of albumen in urine for 17 days longer, at end of which time animal recovered.

   B. A dog received 169 milligrams per kilogram body weight, or 118 grains per 100 pounds body weight; in 24 hours the urine was colored brown; 48 hours after the first dose that dose was repeated, and unconverted color was found in the urine; 48 hours later the same dose was repeated; the color in the urine disappeared in 24 hours; 4 days later the dose was trebled, and the animal vomited, seemed sick for 4 days thereafter; on the fifth day recovery apparently complete. On the sixth day the last dose was repeated, and the animal vomited after the administration. It took no food for 24 hours, and was normal after 48 hours. There was no albuminuria in this case.

   C. A dog received daily for an entire month 45 milligrams per kilogram body weight, or 31½ grains per 100 pounds body weight; it was in good health during the entire time, did not vomit, and ate as usual. Its gain in weight was 6½ per cent.
3. "Bismarck Brown produces, when administered to dogs by the stomach, even in doses of 350 milligrams per kilogram body weight (245 grains per 100 pounds body weight), vomiting and albuminuria. Further disturbance is not noted even in large doses; small doses, 45 milligrams per kilogram body weight (or 31½ grains per 100 pounds body weight), even when frequently administered seem to be entirely harmless. Doses of 16 milligrams are harmless even when introduced into the subcutaneous cellular tissue." (*p. 118.*)
4. "Of the remaining colors some produce vomiting (e. g., Bismarck Brown * * *)." (*p. 147.*)

5. **Lewin** (Lehrbuch der Toxikologie, *1897, p. 231*): Produces eczema, and cites *Deutsch Med. Wochenschr., 1891, p. 45.*
6. **Fraenkel** (*p. 575*): "When the Azo dyestuffs do not contain any sulpho group they are poisonous. Thus, for example, Bismarck Brown * * *. This produces no effect in small doses; on the other hand, doses of 350 milligrams per kilogram of animal (245 grains per 100 pounds) produce albuminuria and vomiting."

DOUBTFUL.

1. **Houghton** (*J. Amer. Chem. Soc., 1907, v. 29, pp. 1351–1357*): Hinders digestion of fibrin, casein and albumen, in strengths of 1:100 or 1:400.

G. T. 201.

Trade names.—Manchester Brown EE; Bismarck Brown R; Vesuvin B.

Names under which it was offered on the United States market as a food color in 1907.—Vesuvin 4 B Conc. Z; Vesuvin B.

Scientific name.—Hydrochlorid of toluene-disazo-meta-tolylene-diamin.

Discovered and patented.—1878.

Shade.—Reddish brown. Offered by 2 out of 12 sources.

UNFAVORABLE.

1. **Lewin** (*Lehrbuch der Toxicologie, 1897, p. 231*): "Produces eczema," and cites *Deutsch. Med. Wochenschr., 1891, p. 45.*

Note.—The literature is not always conclusive as between Nos. 197 and 201, and probably most, if not all, the references under No. 197 also apply to No. 201.

G. T. 240.

Trade name.—Congo Red.

Name under which it was offered on the United States market as a food color in 1907.—Congo.

Scientific name.—Sodium salt of diphenyl-disazo-binaphthionic acid.

Discovered and patented.—1884.

Shade.—Red. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Weyl**: "According to Experiments 1 and 2 Congo Red is, after long-continued administration by the stomach, harmless."
2. A. A dog received 274 milligrams per kilogram body weight, or 192 grains per 100 pounds; this dose was repeated next day, when the urine became pale, was strongly alkaline and contained albumen; the next day the same dose was repeated, whereupon the urine was of a weak red color, and a little albumen present; the next day the dose was increased to 411 milligrams per kilogram body weight, or 298 grains per 100 pounds; the urine was somewhat reddish and contained a little albumen. No color was given for 6 days, at the end of which time the urine was reddish, and deposited a reddish sediment, probably Congo. At the end of that time the dose was increased to 685 milligrams per kilogram

2. A.—Continued.
body weight, or 480 grains per 100 pounds body weight; the urine was feebly alkaline and contained some albumen. The next day the dose last given was doubled; the urine was colored to such an extent that it could be dyed with. Two days later the same dose was repeated; animal took but little food, was otherwise comfortable; there was little albumen present. The loss in body weight was about 4½ per cent. (*p. 141.*)
B. A second dog received 233 milligrams per kilogram body weight, or 163 grains per 100 pounds, daily for one month, and remained entirely well.

G. T. 269.

Trade name.—Chrysamin R.

Name under which it was offered on the United States market as a food color in 1907.—Chrysamin R.

Scientific name.—Sodium salt of ditolyl-disazo-bisalicylic acid.

Discovered and patented.—1884.

Shade.—Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. WEYL (*p. 134*): "The following is a summary of the results obtained with Disazo colors submitted to test by me, viz: Chrysamin R." "All of these proved to be nonpoisonous, * * *."
3. A. A dog received 515 milligrams per kilogram body weight, or 361 grains per 100 pounds; urine became alkaline and yellowish, and easily dyed cotton; very little albumen. Within 24 hours the same dose was repeated, diarrhea resulting and vomiting for 3 days, whereupon the animal was given one-fifth of the dose, or 103 milligrams per kilogram body weight, that is, 72 grains per 100 pounds body weight; the urine continued slightly colored, and contained a distinct amount of albumen, continuing for 2 days, when the last dose was repeated; 24 hours afterwards the dose was doubled, and repeated the next day; the day after the dose was increased 50 per cent; 3 days later the last dose was repeated; slight albuminuria set in, lasting 5 days. B. A dog received three doses of 619 milligrams each per kilogram body weight, or 433 grains per 100 pounds body weight, three times in the course of 10 days; the urine was yellowish in color and contained very little albumen. (*p. 145.*)
4. "Chrysamin is harmless when taken into the stomach." (*p. 147.*)
5. SCHACHERL (*p. 1045*) says Chrysamin is harmless under the conditions in which it is used.

UNFAVORABLE.

1. WEYL (*p. 147*): "Of the remaining colors some produced vomiting * * * others diarrhea (* * * Chrysamin R * * *.")

G. T. 277.

Trade names.—Benzopurpurin 4 B; Cotton Red 4 B; Sultan Red 4 B.

Scientific name.—Sodium salt of ditolyl-disazo-binaphthionic acid.

Discovered.—1884–85.

Shade.—Red. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 130*): On his own experiments classes it as suspicious. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24-hour urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 16	2	8	300	Dog is well, lively; urine normal color; acid; no albumen.
17	....	....	330	Do.
18	3	....	450	Vomiting several times; no albumen.
19	....	....	470	Urine yellow-orange; no albumen; no vomiting.
20	....	....	370	Do.
21	2	....	320	Do.
22	....	....	310	Do.
23	....	....	370	Urine normal; dog is well.
24	....	....	310	Do.
Total.	7			

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 287.

Trade name.—Azo Blue.

Name under which it was offered on the United States market as a food color in 1907.—Azo Blue.

Scientific name.—Sodium salt of ditolyl-disazo-bi-alpha-naphthol-para-sulphonic acid.

Discovered.—1885.

Shade.—Grayish violet. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 134*): The following is a summary of the results obtained with the Disazo colors submitted to test by me, viz: * * * Azo-blue, * * * ." "All these proved to be nonpoisonous."
3. "Azo-blue is harmless, both when administered by the stomach and subcutaneously." A. A dog received 237 milligrams per kilogram body weight, or 166 grains per 100 pounds; 2 days later this same dose was repeated, and the urine was a violet color strongly alkaline and contained a little albumen. The next day the dose was increased to 2.5 times; colorless urine, and little albumen; there was no phenol. The next day 4 times the original dose was administered; abundant bluish-violet urine and little albumen. No administration for 4 days, when 2.5 times the original dose were given, and that dose repeated 3 days later; during this time the urine was colorless and contained little albumen; there was a very slight increase in weight. B. A dog received 319 milligrams per kilogram body weight, or 233 grains per 100 pounds daily for one month; animal remained well with good appetite; a slight amount of albumen made its appearance in the urine. (*p. 144*.)

UNFAVORABLE.

1. WEYL (*p. 144*): "A slight amount of albumen made its appearance in the urine."

G. T. 394.

Trade names.—Dinitrosoresorcin; Dark Green; Russian Green; Alsace Green; Fast Green O; Chlorin; Fast Myrtle Green.

Scientific name.—Dinitroso-resorcinol (Dioximidoquinone).

Discovered and patented.—1875.

Shade.—Green. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. WEYL (*p. 63*): "According to the above experiments Dinitroso-resorcinol is not dangerous to dogs when administered by the stomach even in large doses; while hypodermic administration proves fatal within 24 hours, in the proportion of 190 milligrams per kilogram of body weight" (that is, 132 grains per 100 pounds).
3. A. A dog received 173 milligrams per kilogram body weight, or 121 grains per 100 pounds; scanty dark-brown urine; the next day the same dose was repeated and urine continued dark brown, and contained trace of albumen as well as distinct reaction for iron; the next day the dose was repeated; animal remained lively; the day after that the dose was increased 50 per cent; no albumen. The loss in body weight was only 4 per cent. B. A dog received 198 milligrams per kilogram body weight, or 139 grains per 100 pounds; seems to have been loss of appetite, coupled with dark-brown, almost black urine; 2 days later the dose was doubled, and the urine was colored green by ferrous oxid, and contained no albumen nor sugar; 2 days later the dose was increased 50 per cent, and some albumen was then found in the urine. (*p. 62.*)

G. T. 398.

Trade name.—Naphthol Green B.

Names under which it was offered on the United States market as a food color in 1907.—Naphthol Green; Naphthol Green B.

Scientific name.—Ferrous sodium salt of nitroso-betanaphthol-beta-monosulphonic acid.

Discovered.—1883.

Shade.—Green. Offered by 2 out of 12 sources.

FAVORABLE.

1. WEYL (*p. 64*): A. A dog received 172 milligrams per kilogram body weight, or 120 grains per 100 pounds body-weight; the urine was greenish, and conjunctiva stained intensely green; dose was repeated 3 successive days, and the next day the dose was increased to fivefold; appetite undisturbed, and animal remained lively. B. A dog received 417 milligrams per kilogram body weight, or 292 grains per 100 pounds; there was a dirty yellowish-green color to the urine; no albumen, and not more than traces of iron. The animal was normal within a day, and two days after the same dose was repeated; no change in animal was recorded, except that on the day following the feces were normal, but colored green.

2. **Weyl** (*p. 65*): "Experiments 1 and 2, in which 2 to 5 grams of the color were introduced directly into the stomach, demonstrated its harmlessness in this method of administration."
3. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 65*): "On the other hand, in a hypodermic administration, in two out of three cases abscesses and septic fever were induced."

G. T. 399.

Trade names.—Sun Yellow; Curcumin S; Jaune Soleil; Maize.

Scientific name.—Sodium salt of the so-called Azoxy-stilbene-disulphonic acid.

Discovered.—1883.

Shade.—Yellow. Not offered.

FAVORABLE.

1. **Meyer** (*J. Amer. Chem. Soc., 1907, v. 29, p. 897*): A dog received 100 milligrams per kilogram body weight, or 70 grains per 100 pounds, increased geometrically through the fourth day, when diarrhea set in; up to this time the animal had been given 19.27 grams, or 1,465 milligrams per kilogram body weight, equivalent to 1,026 grains per 100 pounds body weight; the average dose per day would have been 366 milligrams per kilogram body weight, or 256 grains per 100 pounds; the animal was given its fifth portion of coloring matter the same size as the fourth, thereupon color was omitted, and for the following 7 days the dosage of the third day, which amounted to 400 milligrams per kilogram body weight, or 280 grains per 100 pounds body weight, was given; the urine was colored orange throughout the entire test after the first day; the fecal matter also was of orange color; slight diarrhea on the fourth and twelfth days of the test, and vomiting on the fifth day, the cause of which does not seem to have been definitely determined; the autopsy revealed nothing abnormal.

G. T. 425.

Trade names.—Auramin; Auramin O; Pyoctanin Aureum.

Names under which it was offered on the United States market as a food color in 1907.—Auramin O; Auramin; Canary Yellow.

Scientific name.—Hydrochlorid of imido-tetramethyl-diamido-diphenylmethane.

Discovered.—1883.

Shade.—Greenish yellow. Offered by 3 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 157*): On his own experiments classes it as poisonous. The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=69 mg=48 grains.]

Date.	Dose.	Weight.	24-hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Mar. 2-4	1.0	14.4	550	Dog quite normal; acid; no albumen.
6			340	Urine strong brown yellow; traces of albumen; appetite less.
7				Thin stool.
8				Diarrhea; urine brown yellow, pales with sulphuric acid, and contains much albumen.
9				Stool normal; color of urine weaker; much albumen; eats well; lively.
10				Stool normal; urine less colored; less albumen.
11			335	Stool normal; urine less colored; traces of albumen.
12				Stool normal; urine less colored; insignificant traces of albumen.
13				Do.
14	2.0	13.2		Do.
15				Strongly brown yellow; much albumen.
16			None.	Dog depressed; lies down; eats little.
17			295	Vomits and diarrhea; urine strong yellow; little albumen; takes only milk.
19			480	Vomits and diarrhea; urine strong yellow; much albumen.
20				Feces normal color; color urine almost normal; much albumen.
21			352	Feces normal color; color urine almost normal; albumen less.
24	[1] 0.4	13.6	380	Do.

[1] Subcutaneously.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 427.

Trade names.—Malachite Green; New Green; Fast Green; Benzal Green; Diamond Green B; Malachite Green B; New Victoria Green; Vert Diamant; Bitter-almond-oil Green.

Names under which it was offered on the United States market as a food color in 1907.—Green M; New Green Crystals; Green 088.

Scientific name.—Zinc double-chlorid, oxalate, ferric double-chlorid of tetramethyldi-para-amido-triphenyl-carbinol.

Discovered.—1877–78.

Shade.—Bluish green. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 24*): "According to Grandhomme * * * Malachite Green are (is) also nonpoisonous."
3. "* * * Malachite Green are (is) as is now established, almost without poisonous action." (*p. 55.*)
4. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says when free from arsenic it is harmless.
5. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Penzoldt** (*Archiv. exper. path. pharm., 1890, v. 26, p. 312*): One hundred milligrams per kilogram body weight of rabbit, or 70 grains per 100 pounds, injected subcutaneously, caused after the third day motor paralysis and occasional cramps, which resulted fatally at the end of the ninth day.

2. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): In the case of one workman, in contrast with others who had long been unaffected by this substance, itching, burning, inflammation, and swelling of hands and feet, and formation of blisters occurred.

G. T. 428.

Trade names.—Brilliant Green; New Victoria Green; Emerald Green; Malachite Green B; Ethyl Green; Fast Green J.

Names under which it was offered on the United States market as a food color in 1907.—Green E; Green 087; Emerald Green Crystals.

Scientific name.—Sulphate of zinc-double-chlorid (rarely oxalate) of tetraethyl-diamido-triphenyl-carbinol.

Discovered.—1879–80.

Shade.—Yellowish green. Offered by 3 out of 12 sources.

FAVORABLE.

1. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*) says when free from arsenic it is harmless.
2. Buss lists it as nonpoisonous.

UNFAVORABLE.

1. Chlopin (*pp. 171–2*): Classifies it as "very poisonous" on his own experiments (see p. 181). The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=125 mg=87.5 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 24		8	410	Before experiment dog is well; urine normal.
25	2			Soon after giving dye vomiting and diarrhea; in the evening only drank water; vomiting kept up.
26			395	No vomiting; urine greenish; acid; insignificant traces of albumen.
27				Do.
28	2		400	No vomiting; urine greenish; acid; no albumen.
29				Do.
30			395	Do.
May 1				Urine normal; dog quite well.
2				Do.
3			395	Do.
Total....	4			

No. 2.

(1 gram=111 mg=78 grains.)

1902.				
Jan. 21	3	9	400	Before experiment dog quite normal; urine normal; after giving dye very violent vomiting several times.
23–24				Urine greenish in color; acid; very much albumen; no vomiting.
25	2	7.2		Do.
26				Died during the night.
Total....	5			

Experimental data by Chlopin—Continned.

No. 3.

[1 gram=119 mg=83 grains.]

Date.	Dose.	Weight	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 28	3	8.4	370	Before experiment dog and urine normal; soon after giving dye vomiting began, lasting over an hour; dog stands with difficulty.
29	2	....	....	During night dog improved somewhat, began to eat; drinks much; soon after giving dye vomiting.
30	....	....	....	During night vomiting and diarrhea; during night 30th, 31st, in bad condition; does not take food.
31	....	....	....	During night 31st second dog found dead in cage. Cause of death, "paralysis of the heart."
Total....	5			

2. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*): In the case of one workman, in contrast with others who had long been unaffected by this substance, itching, burning, inflammation and swelling of hands and feet, and formation of blisters occurred.

G. T. 433.

Trade name.—Guinea Green.

Name under which it was offered on the United States market as a food color in 1907.—Guinea Green B.

Scientific name.—Sodium salt of diethyldibenzyl-diamido-triphenyl-carbinol-disulphonic acid.

Discovered.—1883.

Shade.—Green. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Chlopin** (*p. 174*): On his own experiments classified it as "nonpoisonous." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=200 mg=140 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 18	2	5	250	Dog and urine normal before experiment.
19	....	....	235	Urine slightly greenish, no albumen; vomited at night.
20	....	....	280	No vomiting.
21	....	....	290	Urine slightly greenish; no albumen.
22	....	....	270	Urine normal in color and composition.
23	....	....	270	Do.
24	2	....	260	Do.
25	....	....	240	Do.
26	2	....	290	Do.
27	....	....	246	Do.
28	2	....	....	Do.
29	....	....	243	Do.
30	2	....	233	Do.
31	....	....	230	Do.
Total.	10			

G. T. 434.

Trade names.—Light Green SF bluish; Acid Green.

Scientific name.—Sodium salt of dimethyldibenzyl-diamido-triphenyl-carbinol-trisulphonic acid.

Discovered.—1879.

Shade.—Green. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*pp. 176–7*): Examined this color, and on his own experiments classes it as "nonpoisonous, but not entirely indifferent." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=133 mg=93 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	7.5	380	Dog and urine normal.
25				Urine green; acid; no albumen.
26			347	Urine green; traces of albumen.
27			297	Do.
28	2			Do.
29				Urine green; no albumen.
30			360	Urine less green; no albumen.
31				Do.
May 1–3				Do.
			370	Do.
Total.	4			

No. 2.

[1 gram=110 mg=77 grains.]

1902.				
Aug. 5	3	9.1	300	Dog and urine normal.
6			310	Urine greenish, acid, no albumen.
7	3			Do.
8	3		320	Do.
9	3		350	Do.
10	3			Do.
11			400	Do.
12		9	350	Do.
Total.	15			

DOUBTFUL.

1. Winogradow (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 435.

Trade names.—Light Green SF yellowish; Acid Green; Acid Green extra conc.

Names under which it was offered on the United States market as a food color in 1907.—Acid Green conc. V N; Light Green SF yellow shade; Acid Green conc. 780; Pistachio.

Scientific name.—Sodium salt of diethyldibenzyl-diamido-triphenyl-carbinol-trisulphonic acid.

Discovered.—1879.

Shade.—Green. Offered by 4 out of 12 sources.

FAVORABLE.

1. Lieber (*p. 144*): The animal was a fully developed male guinea pig, and received per kilogram body weight 240 milligrams, or 168 grains per 100 pounds, five times in all, every other day. There was apparently nothing irregular or abnormal observed during the whole test of nine days.

G. T. 448.

Trade names.—Magenta; Fuchsin; Rosein; Anilin Red.

Obsolete names.—Rubin; Solferino; Fuchsiacin; Rubianite; Azalein; Erythrobenzin; Harmalin.

Names under which it was offered on the United States market as a food color in 1907.—Magenta powder A; Fuchsin Crystals; Magenta F A B S Red 101.

Scientific name.—Mixture of hydrochlorid or acetate of pararosanilin (triamidotriphenylcarbinol) and rosanilin (triamidodiphenyltolylcarbinol).

Shade.—Bluish-red. Offered by 4 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 22*): "The colors examined * * * Fuchsin were (was) found to be nonpoisonous;" "Similarly a hen which had eaten for three weeks oats covered with fuchsin was in good health." (*p. 24.*)
3. "According to Grandhomme rabbits bear without injury fuchsin free from arsenic * * *." (*p. 31.*)
4. "Fuchsin * * * (is) as is now established, almost without poisonous action." (*p. 55.*)
5. Fraenkel (*p. 574*), quoting Penzoldt, says that it is entirely nonpoisonous, and completely prevents putrefaction.
6. Permitted by the law of Austria.
7. Lewin (*Lehrbuch der Toxikologie, 1897, p. 230*), says when free from arsenic it is harmless.
8. Clouet and Bergeron (*J. pharm. chim., 1871, v. 25, p. 296*): One of them took personally 500 milligrams, that is, 7.7 grains in 16 days; there was no digestive disturbance of any kind, and the urine, which was examined daily, contained no albumen. They cite a case of Bright's disease, in which the amount of albumen decreased when fuchsin was administered, and they conclude that fuchsin may be good for sufferers from Bright's disease.

UNFAVORABLE.

1. Forbidden by the law of France. (See *Lieber, p. 31.*)
2. Chlopin (*p. 178*): Examined this color, and on his own experiments classes it as "Suspicious because of vomiting and traces of albumen." The experimental data are as follows:

Experimental data by Chlopin.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 18	3		270	Dog before experiment well and urine normal; vomited several times after receiving dye.
19			280	Urine quite red; acid; no albumen; general condition normal.
20				Do.
21	2		320	Urine less colored; acid; no albumen; vomited once.
22				Urine darkish; acid; traces albumen.
23				Color normal; no albumen.
24				Do.
Total .	5			

NOTE.—This sample may have contained some phosphin, G. T. 532, see page 133.

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 450.

Trade names.—Hofmann Violet; Dahlia; Red Violet 5R extra; Violet R; Iodin Violet; Primula; Violet 5 R; Violet R R.

Scientific name.—Mixture of the hydrochlorids or acetates of the monodi- or trimethyl- (or ethyl-) rosanilins and pararosanilins.

Discovered.—1863.

Shade.—Violet. Not offered.

FAVORABLE.

1. WEYL (*p. 24*): "* * * Anilin Violet (Dahlia) * * * (is) also nonpoisonous."
2. BUSS lists it as nonpoisonous.

UNFAVORABLE.

1. FRAENKEL (*p. 574*) quotes Penzoldt, and says it completely arrests development, and causes muscular paralysis.

G. T. 451.

Trade names.—Methyl Violet B; Direct Violet; Dahlia; Paris Violet; Violet de Methylanilin; Pyoctanin.

Names under which it was offered on the United States market as a food color in 1907.—Methyl Violet; Methyl Violet B; Methyl Violet BB extra; Methyl Violet 3 B D.

Scientific name.—Hydrochlorid of penta- and hexamethyl- pararosanilin.

Discovered.—1861.

Shade.—Violet. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the Austrian law.

3. **Weyl** (*p. 24*): "* * * Anilin Violet (Dahlia) * * * [is] also nonpoisonous."
4. "* * * Methyl Violet [is] as is now established, without poisonous action." (*p. 55*).
5. **Fraenkel** (*p. 573*): "Methyl Violet * * * is relatively nonpoisonous."
6. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Graefe and Braunschweig** (*Fortschr. Medizin, 1890, v. 8, p. 405*): "It seems to be proven that damage will actually result even in the case of most cautious use, which we are sure we exercised."
2. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 52*): I. A dog weighing 7,600 grams received 5.6 grams dye in 12 days; this amounts to 61 milligrams per kilo per day, or 43 grains per 100 pounds per day. Continued vomiting beginning with 0.1 gram dye; progressive emaciation and general falling away; catarrh of eyes and nose; distinct dislike for food and great desire to sleep; temperature below normal, urine unchanged. Loss of weight 1,600 grams, or 21 per cent. The animal died on the thirteenth day. The autopsy showed a pale and blood-poor liver; the kidneys were in a typically congested condition and contained accumulations of blood corpuscles. II. A second dog weighing 6,000 grams received 3.8 grams dye in 14 days, which amounts to 71.4 milligrams per kilo per day, or 50 grains per 100 pounds per day. The animal died on the fourteenth day. There was daily vomiting and rapid emaciation; final weight loss was 1,100 grams, or 18.3 per cent; temperature normal; bloody urine beginning the eighth day. The autopsy showed a blood-poor liver, soft and swollen epithelia; kidneys the same as in the case of the preceding dog.

G. T. 457.

Trade names.—Anilin Blue, spirit-soluble; Spirit Blue; Fine Blue; Bleu Lumière; Opal Blue; Gentian Blue 6B; Hessian Blue; Bleu-de-Nuit.

Scientific name.—Hydrochlorid sulphate or acetate of triphenylrosanilin and triphenylpararosanilin.

Discovered.—1860–1862.

Shade.—Greenish blue; not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 48*) says it is harmless. A dog weighing 4,500 grams received 17 grams dye in 30 days, which amounts to 126 milligrams per kilo per day or 88 grains per 100 pounds per day. Weight remained the same, general condition good, urine and temperature unchanged; killed by chloroform; autopsy showed everything normal.
3. **Lieber** (*p. 14*), where it is stated to be permitted by the Austrian law (*pp. 22–23*), where it is stated to be permitted by the Italian law (*p. 31*), where it is stated to be permitted by the French law in candies, pastilles, sweetmeats, sauces, fruits, and certain liqueurs ordinarily not colored.
4. **Weyl** (*p. 22*), quoting Sonnenkalb (*p. 24*), quoting Grandhomme.
5. **Fraenkel** (*p. 580*) states that it is effective in only 5 per cent of malaria cases.
6. Permitted by the law of Austria.
7. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Weyl** (*p. 23*), quoting Friedrich, where poisoning was produced in a young man engaged in packing this dye.

G. T. 459.

Trade names.—Iodin Green; Pomona Green; Night Green; Vert Lumière.

Scientific name.—Zinc-double-chlorid of heptamethyl-rosanilin-chlorid.

Discovered and patented.—1866.

Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 175*) on his own experiments classes it as "suspicious." (See p. 181). The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 9	6	6	420	Before experiment dog and urine normal.
10			470	Do.
11			441	Urine slightly greenish; no albumen.
12	2		420	Do.
13			390	Urine has greenish opalescence; traces of albumen.
14			400	Do.
15			442	Urine has greenish opalescence; no albumen.
16	2		370	Do.
17				Urine has greenish opalescence; traces of albumen.
18			400	Normal color; no albumen.
Total..	6			

2. **Buss** lists it as poisonous.

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 462.

Trade names.—Acid Magenta; Acid Fuchsin; Acid Rubin; Fuchsin S; Acid Rosein; Rubin S.

Names under which it was offered on the United States market as a food color in 1907.—Acid Magenta Powdered; Acid Magenta.

Scientific name.—Mixture of the sodium or ammonium salts of the trisulphonic acids of rosanilin and pararosanilin.

Discovered.—1877.

Shade.—Red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Cazeneuve (*Arch. gén. méd., 1886, p. 753*) says it may be taken without effect by man and animals, sick or well, in large doses.
3. Weyl (*p. 55*), where he says that it is established of this color that it is almost without poisonous action.
2. Lieber (*p. 14*), where it is stated to be permitted by the law of Austria (*p. 31*), where it is stated to be permitted by the law of France for confectionery, cordials, and the like.
5. Permitted by the law of Austria.
6. Permitted by the law of Italy.
7. Cazeneuve and Lépine (*Compt. rend., 1885, v. 101, p. 1011*): A. Dog: 15 kilos weight, received—

Days.	Grams.	Milligrams per kilo.	Grains per 100 pounds.
15	1	67	47
5	2	134	94
5	5	335	235
5	10	670	470

No diarrhea; no vomiting; no albuminuria; urine colored only occasionally, but did contain the leuco compound of the dye. B. Man: 1. Afflicted with Bright's disease; took two grams daily for one week; no effect. 2. Afflicted with renal cirrhosis; four grams daily for several days; no effect. 3. A well man took four grams daily for several days; no effect.

8. Cazeneuve and Lépine (*Bull. de l'acad. de méd. 1886, p. 643*): Tolerated by man, sick or well.

G. T. 467.

Trade name.—Acid Violet 6B.

Scientific name.—Sodium salt of dimethyl diethyl dibenzyl triamido triphenyl carbinol disulphonic acid.

Discovered and patented.—1889.

Shade.—Violet. Not offered.

FAVORABLE.

1. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 54*): A dog weighing 4,250 grams received 12.5 grams dye in 28 days, which amounts to 105 milligrams per kilo per day, or 74 grains per 100 pounds per day. The dog vomited the color only two or three times and was otherwise normal. Appetite, temperature, and urine all remained normal. Conclusion: Nonpoisonous; autopsy also showed everything normal.

UNFAVORABLE.

Nothing.

G. T. 477.

Trade names.—Alkali Blue; Nicholson Blue; Fast Blue.

Scientific name.—Mixture of sodium salts of triphenyl rosanilin monosulphonic acid and of triphenyl para rosanilin-monosulphonic acid.

Discovered and patented.—1862.
Shade.—Blue. Not offered.

FAVORABLE.

1. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 45*): A dog weighing 4,500 grams received 25 grams dye in 30 days, which amounts to 185 milligrams per kilo per day, or 129.5 grains per 100 pounds per day. The urine remained of normal color, but the stool was a deep blue black. Throughout the whole time the animal was in perfect health. Killed with chloroform; autopsy showed everything normal.

UNFAVORABLE.

Nothing.

G. T. 478.

Trade names.—Bavarian Blue DSF; Methyl Blue, water-soluble; Navy Blue B; Methyl Blue for silk MLB.

Scientific name.—Sodium salt of triphenyl pararosanilin di- and tri-sulpho acid.

Discovered.—1862; not patented.
Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. Chlopin (*p. 170*) examined this color, and on his own experiments classed it as "nonpoisonous but not quite indifferent."

Experimental data by Chlopin.

[1 gram = 115 mg = 81 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 18	2	8.7	450	Dog and urine quite normal.
19			365	Urine slightly greenish; no albumen; acid.
23			380	Do.
24	2		370	Do.
25				Do.
26	2			Do.
27			370	Do.
28	2		320	Do.
29				Do.
30	2			Urine slightly greenish; traces of albumen; acid.
31			525	Do.
June 1				Do.
2			327	Do.
3				Do.
4	3			Do.
5				Urine slightly greenish; traces of albumen; acid; diarrhea.
6	3		405	Do.
7				Urine slightly greenish; traces of albumen; acid.
8	3		395	Do.
9			353	Do.
10	3		370	Do.
11-15			340	Color normal; traces of albumen.
16			[1] 400	Do.
17-29		8.5	425	Do.
Total.	22			

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[1] Daily.

G. T. 479.

Trade names.—Methyl Blue O; Brilliant Cotton Blue greenish; XL Soluble Blue; Diphenylamin Blue; Bavarian Blue DBF; Soluble Blue 8B and 10B; Helvetia Blue.

Scientific name.—Sodium salt of triphenyl-pararosanilin trisulphonic acid.

Discovered and patented.—1862.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 168*) examined this color and on his own experiments classed it as "suspicious" or "nonsuspicious" dependent upon the make of goods.

Experimental data by Chlopin.

No. 1.

[1 gram=75 mg=52 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 14	2	13.4	460	Dog normal; no albumen.
15	....	....	....	Color chocolate-brown; insignificant traces of albumen.
16	2	....	....	Color same; no albumen.
17	....	....	410	Urine normal; no albumen; dog is well.
Total..	4			

No. 2.

[1 gram=152 mg.=106 grains.]

1901.				
Nov. 12	3	6.6	300	Dog quite well; urine normal color; acid; no albumen.
13	....	....	....	Do.
15	3	....	....	Do.
16	3	....	360	Do.
17	3	....	305	Urine scarcely perceptible greenish sheen; acid; no albumen.
19	3	....	330	Urine greenish color; no albumen.
20	....	....	....	Urine darker; no albumen.
21	....	....	....	Urine color normal; no albumen.
22	....	6.8	....	Color almost normal.
Total..	15			

This preparation from Moscow; not suspicious.

No. 3 (MERCK'S PREPARATION).

[1 gram=147 mg=103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.				
Feb. 18	3	6.9	400	Before experiment dog quite normal; urine acid; no albumen; normal yellow color; 2 hours after giving dye vomiting.
19	3	....	....	Vomiting stopped; diarrhea; urine chocolate-brown; reaction acid; considerable albumen; appetite not decreased.
20	3	....	330	During night diarrhea; no vomiting; urine brown; acid; traces of albumen; without acid urine becomes blue.
21	3	....	....	No diarrhea; no vomiting; urine brown; traces of albumen.
22	3	6.8	....	Urine brown, with blue sheen; traces of albumen.
23	....	....	....	Urine blue chocolate-brown; otherwise normal. Urine acquired usual color 8 days later.
Total..	15			

Conclusion: "Suspicious."

G. T. 480.

Trade names.—Soluble Blue; China Blue; Cotton Blue; Bleu Marine; Water Blue; Water Blue 6 B extra; London Blue extra.

Names under which it was offered on the United States market as a food color in 1907.—Pure Soluble Blue.

Scientific name.—Sodium, ammonium or calcium salt of the trisulphonic acid (with some disulphonic acid) of triphenyl-rosanilin and triphenyl-pararosanilin.

Discovered and patented.—1862.

Shade.—Blue. Offered by 1 out of 12 sources.

FAVORABLE.

1. Permitted by law of Austria.
2. Lieber (*p. 147*): A guinea pig received 306 milligrams per kilogram body weight, or 214 grains per 100 pounds, once a day seven times every other day; the weight remained substantially constant, a slight gain of less than ⅔ per cent being noted. "Nothing irregular or disturbing whatsoever was observed during the whole period."
3. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 45*): A dog weighing 4,500 grams received 30 grams dye in 30 days which amounts to 223 milligrams per kilo per day or 156 grains per 100 pounds per day. Animal remained well throughout; no loss of weight or appetite; urine normal color, stool deep blue; killed with chloroform; autopsy showed a pea-green coloring of the cortex of the kidneys.

UNFAVORABLE.

1. Fraenkel (*p. 574*), quoting Penzoldt, says that it completely arrests germ development, and causes changes internally.
2. Santori (*Moleschott's Untersuchungen, 1895, v. 15, p. 46*): Regards this as injurious hypodermically, but not through mouth. A dog weighing 4,000 grams received 3.5 grams of dye hypodermically in 16 days, at the end of which time he died; this amounts to 55 milligrams per kilo per day or 38.5 grains per 100 pounds per day. The autopsy showed the liver to be free from blood; kidneys soft and congested; all organs swollen and colored.

G. T. 483.

Trade names.—Aurin; Rosolic Acid; Yellow Corallin.

Scientific name.—Mixture of aurin (trioxytriphenyl-carbinol) oxidized aurin, methylaurin, and pseudo-rosolic acid (corallin phthalin).

Discovered.—1834.

Shade.—Yellowish brown. Not offered.

FAVORABLE.

1. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): Rosolic Acid is positively nonpoisonous.

UNFAVORABLE.

1. **Chlopin** (*p. 167*) examined this color, and on his own experiments classes it as "strongly poisonous." The experimental data are as follows:

[1 gram=137 mg=96 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	cc.	
Mar. 12	1	7.3	330	Before experiment dog quite well; urine normal color; acid; no albumen.
13	2	....	375	Diarrhea; urine faint red.
14	3	....	300	Diarrhea; lassitude; eats little; urine red; no albumen.
15	3	....	....	Violent diarrhea and vomiting; dog stands on feet with difficulty; eats nothing.
16	....	....	....	Same conditions as on preceding day; dog sick for a long time after.
Total..	9			

2. Prohibited by the German law of 1887.
3. Prohibited by the Belgian law of June 17, 1891.
4. Buss lists it as poisonous.

DOUBTFUL.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*): Rosolic Acid is positively non-poisonous. Small animals can take 1 gram and more of it. In Austria it is prohibited for use in coloring foods. Corallins, or red (Pæonin) or yellow colors, consisting of Aurin and Rosolic Acid were regarded as poisonous because in experiments on man and animals illness occurred, but are said to be poisonous only in the presence of arsenic, phenol, or anilin. These substances are prohibited in the coloring of food.

G. T. 488 or 490.

Trade name.—Victoria Blue; Victoria Blue B; Victoria Blue 4R.

Scientific names.—Hydrochlorid of phenyltetra (penta) methyl-triamido-diphenyl-alpha-naphthyl-carbinol (note: 4R is bracketed).

Discovered and patented.—1883.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 47*): A dog weighing 5,250 grams received 10.5 grams dye in 22 days, which amounts to 45 milligrams per kilo per day or 32 grains per 100 pounds per day. After receiving 1.5 grams the animal suffered copious continuous salivation, anemia, and emaciation and occasional vomiting. The urine's color did not change; the feces became blue; temperature slightly above and below normal; although highly emaciated (loss in weight was 1,750 grams or 33⅓ per cent) the animal retained its appetite; animal died on the morning of the 23d day. The autopsy showed œsophagus, stomach, and intestines colored deep blue and filled with a greenish scum, extended and strong catarrh of the stomach and intestines; kidneys and liver contained very little blood. Conclusion: Poisonous.

(**Note**: It is uncertain which of these two dyes Santori used.)

G. T. 502.

Trade name.—Rhodamin G and G extra.
Scientific name.—Chiefly Triethylrhodamin.
Discovered and patented.—1891.
Shade.—Bluish. Offered by 2 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 184*) examined this color, and on his own experiments classes it as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.	*Grams.*	*Kilos.*	*cc.*	
Feb. 18	3	6	310	Dog normal; urine acid; no albumen; after 2 hours thin stool; remainder of day lively; good appetite.
19	3			Urine fuchsin color, acid, no albumen; stool and appetite normal.
20	3			Goes to stool without results; otherwise as above.
21	3		270	Do.
22	3		320	Do.
23				Goes to stool without results; urine normal after 8 days.
Total..	15			

G. T. 504.

Trade names.—Rhodamin B; Rhodamin O; Safranilin.

Names under which it was offered on the United States market as a food color in 1907.—Rhodamin B extra; Rhodamin; Rhodamin B.

Scientific name.—Hydrochlorid of diethylmeta-amido-phenol-phthalein.

Discovered and patented.—1887.

Shade.—Bluish red. Offered by 5 out of 12 sources.

FAVORABLE.

1. **Lieber** (*p. 141*): A young female rabbit received 339 milligram body weight, or 237 grains per 100 pounds, five times on alternate days. "During the whole period the animal seemed to be perfectly at ease, was lively, displayed good appetite, and gained steadily * * * ." The gain in weight was, roughly, 7 per cent.

UNFAVORABLE.

1. **Chlopin** (*pp. 182, 183*) examined this color, and on his own experiments classifies it as "not poisonous, but not entirely indifferent; suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram = 109 mg = 76 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1904.	*Grams.*	*Kilos.*	*cc.*	
Feb. 18	3	9.2	420	Dog normal; urine acid, no albumen; strong yellow color; 2 hours after giving color thin stool.
19	3	....	400	Urine colored wine red, strong fluorescence, disappears on boiling, and reappears on cooling; no albumen.
20	3	....	400	Thin stool; no change in other respects.
21	3	....	....	No diarrhea; no change in other respects.
22	3	....	420	General condition good; urine red and acid; no albumen.
Total..	15			

Dog recovers.

G. T. 512.

Trade names.—Eosin; Eosin A; Eosin B; Eosin A extra; Eosin Yellowish; Eosin G G F; Water-soluble Eosin; Eosin 3 J and 4 J extra.

Names under which it was offered on the United States market as a food color in 1907.—Erythrosin I N; Eosin J; Eosin Y.

Scientific name.—Alkali salts of Tetra-bromo-fluorescein.

Discovered.—1874.

Shade.—Yellowish red. Offered by 3 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Weyl (*p. 31*): "According to Grandhomme, rabbits bear without injury * * * Eosin * * *."
3. Permitted by the law of Austria.
4. Buss lists it as nonpoisonous.

UNFAVORABLE.

1. Lewin (*Lehrbuch der Toxikologie, 1897, p. 231*): "The continued use of these coloring matters, as well as of Phenolphthalein, which becomes colored in the system, I regard as harmful, and, in fact, through action as coloring matters."
2. Forbidden by the Italian law.

G. T. 516.

Trade names.—Erythrosin G; Pyrosin J; Jaune d'Orient; Dianthin G; Iodeosin G.

Name under which it was offered on the United States market as a food color in 1907.—Erythrosin yellowish shade.

Scientific name.—Sodium or potassium salt of diiodofluorescein.

Discovered.—1875.

Shade.—Yellowish red. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 181*): Examined this color, and on his own experiments reports it as "injurious because of light albuminuria, vomiting, and diarrhea." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram = 156 mg = 109 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	6.4	360	Before experiment dog and urine normal.
25			353	Urine red yellow with greenish sheen; acid; no albumen.
26			368	Urine red yellow with greenish sheen; acid; trace of albumen.
27			370	Do.
28	2			Urine red yellow with greenish sheen; acid; no albumen.
29			355	Do.
30			365	Do.
May 3			365	Do.
Total.	4			

No. 2.

[1 gram = 152 mg = 106 grains.]

1901.				
Nov. 24		6.6	345	Dog and urine normal.
25	3			Do.
26	3			Urine fluorescent orange; no albumen.
27	3			Vomiting, diarrhea; urine orange; no albumen.
28	3		380	No vomiting and no diarrhea; urine fluorescent, red; no albumen.
29	3		340	Do.
30				Do.
Dec. 2-3				Color and composition of urine normal; dog well.
Total.	15			

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 517.

Trade names.—Erythrosin; Erythrosin B; Iodeosin B; Eosin J; Erythrosin D; Pyrosin B; Eosin Bluish.

Names under which it was offered on the United States market as a food color in 1907.—Erythrosin Yellow Shade; Erythrosin B; Erythrosin.

Scientific name.—Sodium or potassium salt of tetraiodofluorescein.
Discovered.—1876.
Shade.—Bluish red. Offered by 5 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.
2. **Weyl** (*p. 31*): "According to Grandhomme, rabbits bear without injury * * * Erythrosine * * *."
3. Permitted by the laws of France.
4. Permitted by the law of Austria.
5. **Fraenkel** (*p. 574*): "Rose Bengal * * * produces no noticeable disturbances."
6. **Buss** lists it as nonpoisonous.

UNFAVORABLE.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*): "The continued use of these coloring matters, as well as of phenolphthalein, which becomes colored in the system, I regard as harmful, and, in fact, through action as coloring matters."

G. T. 520.

Trade names.—Rose Bengal; Rose Bengal A T; Rose Bengal N; Rose Bengal G.

Names under which it was offered on the United States market as a food color in 1907.—Rose Bengal B; Phloxin B.
Scientific name.—Alkaline salt of tetraiododichlorofluorescein.
Discovered.—1875.
Shade.—Bluish red. Offered by 2 out of 12 sources.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 521.

Trade names.—Phloxin; Phloxin TA; Eosin 10B.
Scientific name.—Sodium salt of tetrabromotetrachlorofluorescein.
Discovered.—1882.
Shade.—Red. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the Austrian law.
3. **Chlopin** (*p. 185*) examined this color, and on his own experiments classifies it as "nonpoisonous." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=143 mg=100 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 10	1	7.0	380	Dog and urine quite normal.
11			370	Do.
12			370	Do.
13	3		370	Do.
14				Urine orange.
15			390	Do.
Total..	4			

No. 2.

[1 gram=116 mg=81 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.				
Nov. 3	3	8.6	450	Dog well; urine normal color; acid; no albumen.
4			430	Urine orange; no albumen.
5	3			Do.
6			400	Do.
7	3		440	Do.
8	3		430	Do.
9			450	Do.
10	3		400	Do.
11				Color almost normal; no albumen.
12-14				Do.
Total..	15			

G. T. 527.

Trade names.—Cœrulein S; Alizarin Green; Anthracene Green.
Scientific name.—Sodium bisulphite compound of cœrulein.
Discovered.—1879.
Shade.—Black. Not offered.

FAVORABLE.

1. **Chlopin** (*pp. 186-7*) examined this color, and on his own experiments reports it as "nonpoisonous, and not sufficient data to regard it as suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=147 mg=103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Aug. 30	2	6.8	320	Before experiment dog quite normal.
31			330	Urine normal; acid; no albumen.
Sept. 1	3		300	Do.
2	3		330	Urine has scarcely perceptible sheen; no albumen.
3			300	Do.
4	3			Do.
5	3		300	Do.
6	3			Do.
7	3			Nothing abnormal.
9			310	Do.
10	2			Do.
11			375	Do.
12-18				Do.
Total..	22			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram=167 mg=117 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 3	2	6	270	Before experiment dog quite well; urine normal.
4	3		250	Urine yellow with orange sheen; acid; no albumen.
5	3		265	Thin stool twice; urine dark yellow with greenish sheen; acid; no albumen.
6	2			Stool normal; urine yellow green; acid; no albumen; general condition quite well.
Total..	10			

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 530.

Trade name.—Benzoflavin.

Scientific name.—Hydrochlorid of diamido-phenyl-dimethyl-acridin.

Discovered.—1887.

Shade.—Brownish orange yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 189*) on his own experiments regards this color as suspicious. The experimental data are as follows:

Experimental data by Chlopin.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
May 3	2		365	Before experiment dog well and urine normal.
4			391	Dog ate poorly; traces of albumen.
5	2			Do.
6			330	Urine greenish sheen; acid.
7	2		375	Color same; no albumen.
8				Do.
9				Do.
Total..	6			

G. T. 532.

Trade names.—Phosphin; Xanthin; Leather Yellow; Philadelphia Yellow G.

Scientific name.—Nitrate of chrysanilin (unsym. diamido-phenyl-acridin) and homologues.

Discovered.—1862.

Shade.—Orange yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 190*) reports Fuchsin containing Phosphin as suspicious.
2. On his own experiments says this color "does not belong to the poisonous colors, but is not wholly harmless." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram—147 mg—103 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	cc.	
Apr. 15		6.8	390	Dog well; urine normal.
16	2		350	Do.
17			350	Do.
18	3			Urine yellower than normal; vomited several times after taking dye; no albumen.
19			330	Urine same; no vomiting.
21	2		370	Vomited twice; urine same.
22				No vomiting; urine same.
23				Color normal.
24				Dog well; urine normal.
Total..	7			

3. (*p. 178*): See experimental data on G. T. 448, which also applies to this color.
4. **Fraenkel** (*p. 578*): Where its physiological action is compared with quinin its action on protozoa is far greater. "The Phosphins are locally strong irritants, and producers of inflammation of medium poisonous nature so that humans can very well bear 400 milligrams, or 6.17 grains.
5. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Phosphin * * * produces in humans, in doses up to 1 gram, vomiting and diarrhea."

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 563.

Trade names.—Alizarin Blue S; Anthracene Blue S; Alizarin Blue ABS.

Scientific name.—Sodium bisulphite compound of dioxyanthraquinone-beta-quinolin.

Discovered and patented.—1881.

Shade.—Blue. Not offered.

FAVORABLE.

1. Permitted by law of Austria.

UNFAVORABLE.

1. **Chlopin** (*p. 171*): On authority not given reports this color as poisonous or harmful.
2. **Ehrlich** (*Das Sauerstoffbeduerfniss des Organismus, 1885, p. 23*): "Per kilogram of rabbit, 12–15 cc of this solution in general produce death within the first quarter of an hour; whereas 4 cc of the same did not usually produce it, and 7 cc represent a medium, when properly applied, fatal dose." (This solution contained not to exceed 17 per cent coloring matter; each cubic centimeter represents 170 milligrams per kilogram body weight, or 119 grains per 100 pounds; the coloring matter was introduced subcutaneously.)
3. **Buss** lists it as poisonous.

G. T. 572.

Trade name.—Indophenol white; Leucindophenol.

Scientific name.—Tin compound of dimethyl-para-amido-phenyl-para-oxy-alphanaphthylamin.

Discovered.—1881.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 57*): A dog weighing 4,000 grams received 18 grams dye in 30 days, which amounts to 150 milligrams per kilo per day or 105 grains per 100 pounds per day. Temperature, urine, and weight all remained unchanged. Animal killed with chloroform; autopsy showed fatty degeneration of the liver; everything else normal. (Santori classes this color as "not nonpoisonous.")

G. T. 574.

Trade names.—Ursol D; Ursol P; Ursol DD.

Scientific name.—Hydrochlorids of para-phenylene diamin, para-amidophenol, and diamidodiphenylamin, respectively.

Discovered.—1888.

Shade.—Brown to black. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 214*) examined this color, and on his own experiments reports it as "strongly poisonous." On January 15, 1901, a dog weighing 18.4 kilos was given 3 grams. An hour or an hour and a half after giving dye vomiting set in; dog lay down on one side and died in 3 or 4 hours. Cause of death, heart filled with coagulated blood; lungs, liver, and kidneys filled with blood; turbid swelling of the liver and heart; mucous membrane of stomach inflamed; brain unchanged. Death caused by paralysis of the heart. It also acts severely on the skin.

G. T. 576.

Trade names.—New Gray; Malta Gray; Nigrosin; Direct Gray; Methylene Gray; New Methylene Gray.

Scientific name.—(?)

Discovered.—1888.

Shade.—Gray. Not offered.

FAVORABLE.

1. CHLOPIN (*p. 209*) examined this color, and on his own experiments concludes it contains "no poisonous properties." The experimental data follow:

Experimental data by Chlopin.

[1 gram=77 mg=54 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Feb. 8	3	12.9	510	Dog quite well; urine quite normal.
9			480	Urine green color; acid; no albumen.
10	3		400	Do.
11	3		450	Do.
12	3		475	Do.
13	3			Vomited once; urine dark green; acid; no albumen.
14	3			Do.
15	3	12.8	490	Do.
16				Urine lighter shade.
17			475	Urine normal; dog well.
Total.	21			

G. T. 584.

Trade names.—Safranin; Safranin S; Safranin conc.; Safranin GOO; Safranin T; Safranin extra G; Safranin FF extra; Safranin AG, AGT, and OOF.

Name under which it was offered on the United States market as a food color in 1907.—Safranin SP.

Scientific name.—Mixture of diamido-phenyl-and-tolyl-tolazonium chlorids.

Discovered and patented.—1859.

Shade.—Reddish brown. Offered by 1 out of 12 sources.

FAVORABLE.

1. Cazeneuve (*Arch. gén. de méd. 1886, Vol. I, p. 753*): Produces gastro-intestinal disturbances but is not a violent poison.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. Weyl (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of * * * Safranine."
3. "This body (Chamber of Commerce of Sonneberg) recommends for the preparation of children's toys three colors, the poisonous character of which I can demonstrate. These are * * * Safranine * * * . (*p. 34.*)
4. Weyl (*Handbuch der Hygiene*): "According to Theodore Weyl this is even in small doses, when injected subcutaneously, a strong poison" (50 milligrams per kilo body weight, or 35 grains per 100 pounds); "whereas, when administered by the stomach only large doses over a long period of time produce diarrhea."
5. Lewin (*Lehrbuch der Toxikologie, 1897, p. 232*): "Safranin is poisonous when injected intravenously. (Pulse acceleration, dyspnœa, cramps.) Fed to dogs by the mouth it causes only diarrhea."
6. Prohibited by the Resolutions of the Society of Swiss Analytical Chemists, of September, 1891.
7. Prohibited by the Canton of Tessin.
8. Buss lists it as poisonous.
9. Cazeneuve and Lépine (*Compt. rend. 1885, v. 101, p. 1011*): A dog (weight not given) was given, by the mouth, daily doses of from 1 to 4 grams for several weeks; only salivation and diarrhea were produced. They conclude that it is a harmful color.

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 593.

Trade names.—Mauve; Mauvein; Chrome Violet.

Obsolete names.—Mauve Dye; Anilein; Anilin Purple; Violein; Indisin.

Scientific name.—Salts of phenyl- and tolyl-safranins.

Discovered and patented.—1856.

Shade.—Blue, Reddish Blue, Bluish Violet. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 599.

Trade names.—Indulin, spirit soluble; Indulin opal; Fast Blue R and B, spirit soluble; Indulin 3B and 6B, spirit soluble; Indulin 3B opal; Indulin 6B opal; Azin Blue, spirit soluble; Indigen D and F; Printing Blue; Acetin Blue.

Scientific name.—Mixtures of dianilidoamido, trianilido and tetra-anilido phenyl-phenazonium chlorids.

Discovered and patented.—1863.

Shade.—Blue. Not offered.

FAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895*, v. *15*, *p. 50*): A dog weighing 4,600 grams received 12.5 grams of this dye (in oil) in 30 days, which amounts to 91 milligrams per kilo per day, or 64 grains per 100 pounds per day. There was no disturbance of any kind. Killed by chloroform; autopsy showed everything to be normal.

G. T. 600.

Trade names.—Nigrosin, spirit soluble; Coupier's Blue; Oil Black; Sloelin; Spirit Black.

Scientific name.—Mixtures of Indulins with allied bases and fluorindins.

Discovered and patented.—1867.

Shade.—Black. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.

G. T. 601.

Trade names.—Indulin, soluble; Indulin 3B; Fast Blue R and 3R; Sloelin R S and B S; Indulin R and B; Indulin 6 B; Fast Blue 2R, B, and 6B.

Scientific name.—Mixtures of the sodium salts of the sulphonic acids of the various spirit-soluble indulins.

Discovered and patented.—1867.

Shade.—Bronzy or Blue Black. Offered by 1 out of 12 sources.

FAVORABLE.

1. **Chlopin** (*pp. 198–9*): On his own experiments classifies this color as nonpoisonous. The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram = 54 mg = 38 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 5	3	18.4	650	Dog and urine normal.
6			670	Urine brown; acid; no albumen.
7	3		400	Urine greenish brown; no albumen.
8	3		410	Do.
9	3		400	Do.
10	3		400	Do.
11	3		500	Do.
12			560	Do.
13			650	Do.
14			620	Color and composition of urine normal; dog is well.
Total.	18			

No. 2.

[1 gram = 114 mg = 80 grains.]

1902.				
Jan. 14	3	8.8	420	Dog and urine normal.
15	3		390	Urine slightly blue; acid; no albumen.
16	3			Do.
18			420	Do.
19				Urine almost normal color; no albumen.
20				Color and other properties of urine normal.
21	3	8.8		No record.
22	3		400	Urine slightly bluish; no albumen.
23	3		460	Do.
24			400	Dark green; no albumen.
25			450	Feeble green; no albumen.
26			400	Urine normal; dog well.
Total.	18			

UNFAVORABLE.

1. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 55*): A dog weighing 4,500 grams received 4 to 6 grams dye in 7 days, which amounts to 127 to 190 milligrams per kilo per day, or 89 to 133 grains per 100 pounds per day. No vomiting. Stool black blue and no change in the urine. Up to the sixth day the animal, in very good general condition, ate heartily and was quite lively; temperature unchanged. On the morning of the sixth day the animal was found in his cage suffering from general muscular cramps which were heightened by the slightest noise; the animal did not respond to calls or threatening movements and was in a complete stupor. This continued for 24 hours, when the animal died. The autopsy showed numerous punctures of the lungs and of the mucous membrane of the stomach; fatty degeneration of the liver and little blood in it; kidneys without change, although the cortex was colored a light green.

G. T. 602.

Trade names.—Nigrosin, soluble; Bengal Blue; Gray R and B.

Scientific name.—Sodium salts of sulphonic acids of spirit nigrosins.

Discovered and patented.—1867.

Shade.—Black. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 231*) says: "Produces eczema," and cites *Deutsche. Med. Wochenschr., 1891, page 45.*

G. T. 614.

Trade names.—Magdala Red; Naphthalene Rose; Sudan Red; Naphthalene Red; Naphthylamin Pink.

Scientific name.—Mixture of amido-naphthyl-naphthazonium-chlorid and diamido-naphthyl-naphthazonium chlorid.

Discovered.—1868.

Shade.—Red. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 200*) **examined this color, and on his own experiments concludes it "does not belong to the poisonous list, but is not wholly harmless." The experimental data are as follows:**

Experimental data by Chlopin.

No. 1.

[1 gram=91 mg=64 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Apr. 12	2	11	780	Before experiment dog and urine normal.
13			1,100	Urine rose-colored; acid; no albumen.
14	3		760	Do.
15			660	Urine quite red; acid; no albumen; vomited once.
18			670	No vomiting; dog is well; urine normal in color and composition.
Total .	5			

No. 2.

[1 gram=111 mg=78 grains.]

1903.				
Feb. 3	3	9	380	Day before experiment quite well; urine normal color; acid; no albumen.
4	3		340	In the morning three times thin stool, urine faintly rose-colored; acid; no albumen.
5	3		370	No diarrhea; urine rose-red; acid; no albumen.
6				Do.
7	3			Do.
8	3		310	Do.
9			320	Do.
Total .	15			

Experimental data by Chlopin—Continued.

[1 gram=114 mg=80 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.	*Grams.*	*Kilos.*	*cc.*	
Feb. 10	3	....	310	Vomiting and diarrhea; urine rose-red; no albumen; acid; with muriatic acid rose color, becomes greenish gray.
11	....	....	315	No vomiting; no diarrhea; urine rose-red; acid; no albumen.
12	....	8.8	350	No vomiting; no diarrhea; color reaction of urine normal; no albumen.
Total.	3			

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm. 1903, v. 6, p. 589*) says it almost completely inhibits digestion.

G. T. 620.

Trade names.—Gallocyanin DH and BS; Fast violet; Gallocyanin RS, BS, and D.

Scientific name.—Dimethylamido dioxy phenazoxoniumcarboxylate. (BS is the bisulphite compound.)

Discovered and patented.—1881.

Shade.—Bluish Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 51*): A dog weighing 5,400 grams received 7.5 grams dye in 30 days, which amounts to 46 milligrams per kilo per day or 32 grains per 100 pounds per day. Throughout all the time the animal remained well, had good appetite, temperature normal, no loss of weight; urine and feces colored deep blue black. Killed by chloroform. Autopsy showed incipient fatty degeneration of the liver and a swelling of the kidneys. Conclusion: Poisonous.

G. T. 639.

Trade names.—Meldola's Blue; Cotton Blue R; Fast Navy Blue R; Naphthol Blue R and D; Naphthylene Blue R in crystals; Fast Blue R, 2 R and 3 R for cotton in crystals; Fast Navy Blue RM and MM.

Scientific name.—Zinc double chlorid of dimethylamido-naphthophenoxazonium chlorid.

Discovered.—1879.

Shade.—Dark Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*pp. 194-195*) on his own experiments reports this color as "very poisonous." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=68 mg=48 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Jan. 5...	3	14.6	590	Before experiment dog quite well; urine normal in color and composition; 1 hour after giving the dye vomiting began; dog lay down on ground and died in 1 hour.
Total.	3			

No. 2.

[1 gram=167 mg=117 grains.]

1902.				
Jan. 14	3	6	....	Soon after giving dye, strong vomiting and diarrhea; after a few hours improved.
15	3	....	....	Most violent vomiting: dog lay down on his side, and was found dead in 6 hours.
Total.	6			

NOTE.—See p. 181.

2. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 49*): A dog weighing 4,500 grams received 12.5 grams dye during 30 days; this amounts to 93.3 milligrams per kilo per day or 65.3 grains per hundred pounds per day. Continued vomiting beginning with 0.2 grams dye, anaemia and copious as well as continuous salivation and emaciation; the animal lost 1,200 grams in weight or 27 per cent. Killed by chloroform; autopsy showed no fat, flabby muscles, stomach contracted and filled with mucous and in part colored pea green; fatty degeneration of the liver; contracted bladder; kidneys swollen and congested with blood and decomposed blood corpuscles in the Bowman capsules.

G. T. 649.

Trade names.—Gentianin; Gentiana Violet.

Scientific name.—Zinc double chlorids of dimethyldiamido phenazthionium chlorid.

Discovered and patented.—1886.

Shade.—Violet. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 54*): A dog weighing 3,000 grams received 4.7 grams of dye in 7 days, which amounts to 224 milligrams per kilo per day or 157 grains per 100 pounds per day. Beginning with the third day the dog appeared weak and depressed and a whitish froth appeared at the mouth; mild diarrhea and complete aversion to food; temperature and urine unchanged. Died on seventh day. Autopsy showed congestion of mucous membrane of stomach; the liver was inflamed and the kidneys strongly congested.

G. T. 650.

Trade names.—Methylene Blue B and BG; Methylene Blue BB in powder extra D; Methylene Blue BB in powder extra; Methylene Blue A extra.

Names under which it was offered on the United States market as a food color in 1907.—Methylene Blue B; Methylene Blue.

Scientific name.—Chlorid or zinc double-chlorid of tetramethyldiamido-phenazthionium.

Discovered.—1876.

Shade.—Blue. Offered by 2 out of 12 sources.

FAVORABLE.

1. **Schacherl** (*p. 1046*): "To these groups belong the much-used Methylene Blue, which in moderate doses is harmless."
2. **Fraenkel** (*p. 574*): "* * * Methylene Blue causes no noteworthy disturbances."
3. **Cazeneuve** (*Arch. gén. de. méd. 1886, v. 1, p. 753*) says that it produces gastric intestinal derangements but is not a violent poison.

UNFAVORABLE.

1. Prohibited by Confectioners' List.
2. **Weyl** (*p. 31*): "Cazeneuve and Lépine pointed out the poisonous nature of * * * Methylene Blue * * *."
3. **Fraenkel** (*p. 579*): "To regard Methylene Blue as a specific remedy such as quinin, is, in spite of a few such experiments, improper; it produces subsidiary effects which depend in part upon local irritation of the intestinal tract, and partly, however, upon irritation of the bladder with increased micturition."
4. **Cazeneuve** (*Arch. gén. de méd. 1886, v. 1, p. 753*), says that it produces gastric intestinal derangements.
5. **Combemale** and **Francois** (*Sem. Med. 1890, no. 31, p. 258*), say that it produces intestinal disorders and vomiting, colored urine, and colored feces in dogs, and therefore is a highly injurious color.
6. **Santori** (*Moleschott's Untersuchungen, 1895, v. 15, p. 42*) classes it as injurious. A dog weighing 4,600 grams received in 20 days 18 grams dye, which amounts to 196 milligrams per kilo per day, or 137 grains per 100 pounds per day. Urine and feces colored; diarrhea and continuous vomiting; blood and pus contained in stool; loss of appetite; loss of weight was 1,600 grams, or 35 per cent. Animal died. Autopsy disclosed blue-colored skin and fat; brain turned blue on exposure to air, but only the outer cortex was colored; stomachical catarrh; the mucous membrane of the stomach colored blue; the heart sac and the pleura colored blue to blue-green; intestines externally blue; kidneys were thickened and colored dark blue throughout; parenchymatous kidney inflammation; fatty degeneration of the liver; diaphragm locally colored.
7. **Arloing** and **Cazeneuve**, quoting Cazeneuve and Lépine (*Arch. de méd. v. 9, p. 364*), say that it is not inactive.
8. Forbidden by Resolutions of Swiss Analytical Chemists, September, 1891.

9. **Galliard** (*Rev. intern. des falsifications; abst. Hygien. Runsdach. 1892, p. 104*): "Methylene Blue, which is frequently used for coloring foods, can cause, even in small doses (10 to 20 milligrams, or one-sixth to one-third of a grain), a feeling of general depression, nausea, and certain feelings of pain, and can even produce transitory albuminuria. In larger doses (40 to 60 milligrams, or six-tenths to nine-tenths of a grain), it causes in the case of persons not accustomed thereto, vomiting, diarrhea, increased micturition, and albuminuria. In the case of persons suffering from nervous diseases, it frequently produces disturbance which has as its consequence a cessation or a change of place of the pain. Sometimes it produces painlessness, or an easing of pain in the patient, for which no certain therapeutic indication could be determined."

10. **Weyl** (*Handbuch der Hygiene*) comments as follows: "These statements of Galliard arouse but little confidence, because, as is well known, Methylene Blue is very frequently administered to invalids in doses of more than 0.5 gram without any noticeable disturbance. Perhaps Galliard's preparation was unclean. At any rate, in all experiments on the poisonous nature of Methylene Blue it is to be considered that it frequently occurs in commerce as a zinc chlorid double salt."

11. **Lewin** (*Lehrbuch der Toxikologie, 1897, p. 232*): "Methylene Blue * * * can produce after prolonged administration of 0.5–1.5 gram daily increased micturition, irritation of the bladder, blue coloration of the urine, and saliva, diarrhea, headaches, vertigo, delirium, and twitching of the muscles, the latter symptoms probably because the coloring matter is deposited in the brain."

12. **Buss** lists it as poisonous.

G. T. 651.

Trade name.—Methylene Green G conc. extra yellow shade.
Scientific name.—Nitromethylene Blue.
Discovered and patented.—1886.
Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*pp. 192–193*) examined this color, and on his own experiments concludes that it is "nonpoisonous, but somewhat suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=71 mg=50 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Apr. 24	2	14	580	Dog well; urine normal.
25				Urine dark green; acid; no albumen.
26				Urine less colored; acid; traces of albumen.
28	2			No record.
29				Urine dark brown; no albumen; acid.
30			485	Do.
May 2				Color normal; acid; no albumen.
Total.	4			

Experimental data by Chlopin—Continued.

No. 2.

[1 gram=122 mg=85 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Nov. 25	3	8.2	430	Dog well; urine normal.
26	3	....	445	Urine strongly green; no albumen.
27	3	....	450	Do.
28	3	....	410	Diarrhea; urine green; acid; no albumen.
29	3	....	440	No diarrhea; urine same.
Dec. 1-2	....	....	....	Color normal; acid; no albumen; dog lively and well.
Total.	15			

DOUBTFUL.

1. **Winogradow** (*Zts. Nahr. Genussm., 1903, v. 6, p, 589*), says it noticeably retards digestive action; is not indifferent.

G. T. 654.

Trade name.—Toluidin Blue O.

Scientific name.—Zinc-double-chlorid of dimethyl-diamido-tolu-phenazthonium-chlorid.

Discovered and patented.—1888.

Shade.—Blue. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Fraenkel** (*p. 574*): "Toluidin Blue * * * is a strong poison for micro-organisms, and may be used in eye treatment, like Methylene Blue."

G. T. 659.

Trade names.—Primulin; Thiochromogen; Sulphin; Polychromin; Aureolin.

Scientific name.—Sodium salt of the mono-sulphonic acids of the dehydrothionated condensation products of dehydrothiotoluidin (mixed with some sodium dehydrothiotoluidin-sulphonate).

Discovered.—1887.

Shade.—Yellow. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. **Chlopin** (*p. 203*) examined this color, and on his own experiments reports it as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=154 mg=108 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 21	2.0	6.5	292	Dog and urine normal.
22			294	Urine dark brown; insignificant traces of albumen; acid.
23	3.0		355	Color normal; no albumen.
24	[1] 0.5	6.3	260	Urine dark brown; acid; no albumen.
25				Do.
26				No albumen; dark-brown color; dog has lassitude; does not eat bread nor take milk, only a little meat.
27			291	No albumen; dark-brown color; dog has lassitude; dog eats little.
28				At the point of injection an abscess appears; urine acid; no albumen; yellow color.
29			270	Urine acid; no albumen; yellow color; dog eats more.
30			295	Urine acid; no albumen; yellow color; abscess broke.
Apr. 2			280	Urine normal; appetite almost normal.
Total.	[1] 5.0 [2] 0.5			

[1] By mouth.

[2] Subcutaneously.

2. See also CHLOPIN (*Zts. Nahr. Genussm., 1902, v. 5, p. 241*).

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 667.

Trade names.—Quinolin Yellow; Quinolin Yellow, water-soluble.

Name under which it was offered on the United States market as a food color in 1907.—Chinolin Yellow O.

Scientific name.—Sodium salt of the sulphonic acid (chiefly disulphonic acid) of quinophthalone.

Discovered.—1882.

Shade.—Greenish Yellow. Offered by 1 out of 12 sources.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 205*) on his own experiments reports this color as "suspicious." The experimental data are as follows:

Experimental data by Chlopin.

No. 1.

[1 gram=44 mg=31 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 19	3.00	22.6	730	Before experiment dog and urine normal.
20			720	Urine dark brown; traces albumen; acid.
21			749	Color of urine normal; no albumen.
22				Do.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Mar. 23	0.75	21.4	750	Urine dark brown; no albumen.
24				Dog eats nothing.
25			693	Do.
26			725	Do.
27				Dog eats nothing; eats poorly; albumen in urine.
28				Dog eats nothing; rather much albumen.
29				Do.
30				Do.
Apr. 2				Dog eats nothing; traces of albumen.
4	[1]3.00		710	Color of urine almost normal; insignificant traces of albumen.
5				Urine yellow brown; traces of albumen; appetite normal.
6				Urine yellow brown; no albumen.
8			700	Urine and dog normal.
Total..	[1]6.00 [2]0.75			

No. 2.

[1 grain=133 mg=83 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1903.				
Mar. 3	3.00	7.5	320	Before experiment dog quite well, and urine normal.
4	3.00		300	Color dark yellow; no albumen; general condition normal.
5	3.00		300	Color yellower than normal; acid; no albumen.
6	2.00	7.4		Do.
Total..	11.00			

[1] By mouth.

[2] Subcutaneously.

DOUBTFUL.

1. WINOGRADOW (*Zts. Nahr. Genussm., 1903, v. 6, p. 589*) says it noticeably retards digestive action; is not indifferent.

G. T. 670.

Trade names.—Vidal Black; Vidal Black S.

Scientific name.—Possibly the sulpho-hydro derivative of a polythiazin.

Discovered.—1893.

Shade.—Green. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*p. 208*) examined this color, and on his own experiments classes it as "very harmful." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=132 mg=92 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 29	2	7.6	386	Before experiment dog quite normal; soon after taking dye vomited twice.
30	3		390	General condition good; no vomiting; urine turbid, alkaline; no albumen.

Experimental data by Chlopin—Continued.

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1901.	*Grams.*	*Kilos.*	*cc.*	
Dec. 31	3			Vomited soon after getting dye; appetite less; no albumen.
1902.				
Jan. 1	3			Urine turbid; scarcely noticeable blackish sheen; green with sulphuric and hydrochloric acid.
2		7	369	Do.
4	3		370	Vomited all day after taking dye; eats little; no albumen.
5	3			Violent and prolonged vomiting and diarrhea; condition pitiable; further giving of dye stopped, so as not to kill the animal.
6-7				Vomiting and diarrhea continue.
8				Vomiting and diarrhea stopped.
9-11				Dog is livelier, and begins to eat.
13				Dog looks well; urine normal in color; acid; no albumen.
Total..	17			

G. T. 675.

Trade names.—Thiocatechin; Thiocatechin S.
Shade.—Brown. Not offered.

FAVORABLE.

Nothing.

UNFAVORABLE.

1. CHLOPIN (*pp. 210, 211*) examined this color and classes it as "very poisonous." The experimental data are as follows:

Experimental data by Chlopin.

[1 gram=128 mg=90 grains.]

Date.	Dose.	Weight.	24 hours' urine.	General condition of animal and urine.
1902.	*Grams.*	*Kilos.*	*cc.*	
Feb. 13	2.4	7.8	300	Before experiment dog and urine quite normal; 10 or 15 minutes after giving dye dog fell on one side, limbs extended, stomach drawn in; small and frequent convulsions; retching; then abundant vomiting, same color as dye; soon after vomiting dog got up and walked as if drunk; hind legs tend to collapse; salivation.
14	2.0		290	A few minutes after giving dye, dog again fell as if in an epileptic fit; convulsions of the extremities, which soon ceased, but the dog still lay stretched out, with open eyes, which reacted to light; tongue hanging out to one side; after 10 or 15 minutes vomiting began; dog still lying on one side, assumed a more normal attitude; 15 minutes later dog got up, walked as if drunk; poor control over hind legs.
15	0.5			Vomits at once after getting dye, but remained standing.
16				Dog appears depressed, but eats.
18				Dog quite well; urine usual normal color; no albumen.
Total..	4.9			

G. T. 689.

Trade names.—Indigo; Indigo Pure BASF.
Scientific name.—Indigotin.
Shade.—Blue. Not offered.

FAVORABLE.

1. Permitted by Confectioners' List.
2. Permitted by the law of Italy.

UNFAVORABLE.

1. FRAENKEL (*p. 571*): "However, pure Indigo, according to Kobert, is, in finely divided condition, a violent local irritant."

G. T. 692.

Trade names.—Indigo Carmine; Indigo Extract; Indigotin.

Names under which it was offered on the United States market as a food color in 1907.—Indigo Carmine Powder IN; Indigotin; Indigotin A.

Scientific name.—Sodium salt of Indigotin disulphonic acid or the free acid.

Discovered.—1740.

Shade.—Blue. Offered by 3 out of 12 sources.

FAVORABLE.

1. SCHACHERL (*p. 1046*): "No objection to its use."
2. SANTORI (*Moleschott's Untersuchungen, 1895, v. 15, p. 41*): A dog weighing 4,500 grams received 90 grams dye in 30 days; vomited twice during the examination; no change in weight; animal killed with chloroform; autopsy showed slight dull swelling in the epithelium and convoluted canals of the kidneys. This dosage amounts to 667 milligrams per kilo per day, or 467 grains per 100 pounds per day. Classes it as harmless.

ALPHABETICAL INDEX OF TRADE NAMES OF COAL-TAR COLORS.

The following list of the trade names of coal-tar colors appearing in the foregoing compilation on physiological action is complete when supplemented by the list of 23 colors given on page 227; Green Table numbers in parenthesis.

Acetin Blue (599).
Acid Brown (138).
Acid Green (434, 435).
Acid Green ex. conc. (435).
Acid Green conc. (435).
Acid Green conc. VN (435).
Acid Green conc. 780 (435).
Acid Fuchsin (462).
Acid Magenta (462).
Acid Magenta powd. (462).
Acid Rosein (462).
Acid Rubin (462).
Acid Violet (467).
Acid Violet 6 B (467).
Acid Yellow (8).
Acid Yellow AT (94).
Acid Yellow D (88).
Acid Yellow G (8).
Acid Yellow G pat. (8).
Acme Yellow (84).
Alizarin Blue ABS (563).
Alizarin Blue S (563).
Alizarin Green (527).
Alphanaphthol Orange (85).
Alsace Green (394).
Amaranth (107).
Amaranth B (107).
Anilein (593).
Anilin Blue Sp. Sol. (457).
Anilin Orange (2).
Anilin Purple (593).
Anilin Red (448).
Anilin Yellow (4).
Anilin Yellow S (4).

Anthracene Green (527).
Anthracene Blue S (563).
Archil Substitute V (28).
Atlas Orange (86).
Auramin (425).
Auramin O (425).
Aurantia (6).
Aureolin (659).
Aurin (483).
Azalein (408).
Azarin S (70).
Azin Blue, spirit soluble (599).
Azo Acid Rubin (103).
Azo Acid Yellow (92).
Azo Blue (287).
Azo Flavin (92).
Azo Fuchsin G (93).
Azo Rubin (103).
Azo Rubin A (103).
Azo Rubin S (103).
Bavarian Blue DBF (479).
Bavarian Blue DSF (478).
Bengal Blue (602).
Bengal Green (427).
Benzoflavin (530).
Benzopurpurin 4B (277).
Betanaphthol Orange (86).
Biebrich Scarlet (163).
Bismarck Brown (197).
Bismarck Brown R (201).
Bitter-almond-oil Green (427).
Bleu de Nuit (457).
Bleu Lumière (457).
Bleu Marine (480).
Bordeaux B (65).
Bordeaux B L (65).
Bordeaux R ext. (65).
Bordeaux S (107).
Brilliant Black B (188).
Brillant Cotton Blue, greenish (479).
Brilliant Green (428).
Brilliant Orange (13).
Brilliant Scarlet (106).
Brilliant Scarlet 4R (106).
Brilliant Yellow (5).
Brilliant Yellow S (89).
Butter Yellow (16).
Canary Yellow (425).
Carbazotic Acid (1).
Cardinal 3B (103).
Carminaph (11).
Carmoisin (103).
Carmoisin B (103).
Cerasin (102).
Cerasin Orange I (11).
China Blue (480).
Chlorin (394).
Chrome Violet (593).
Chrysamin R (269).
Chrysaurein (86).
Chryseolin (84).
Chrysoidin Crystals (17, 18, 41).
Chrysoidin R (17, 18, 41).
Chrysoidin Y (17, 18, 41).
Chrysoin (84).
Chrysoin REZ (84).
Cinnamon Brown (197).
Citronin (4).
Claret Red (65).
Claret Red RZ (103, 106, 107).
Cœrulein S (527).
Cochineal Red A (106).
Congo Red (240).
Cotton Blue (480).
Cotton Blue R (639).
Cotton Red 4B (277).
Coupier's Blue (600).
Crocein Orange (13).
Crocein Orange 10234 (13).
Crocein Orange G (13).
Crocein Scarlet (106).
Crocein Scarlet 3B (160).
Crocein Scarlet 4BX (106).
Crocein Scarlet 7B (169).
Crocein Scarlet 8B (169).
Curcumin (89).
Curcumin S (399).
Dahlia (450, 451).
Dark Green (394).
Diamond Green B (427).
Dianthin G (516).
Dinitro-cresol (2).
Dinitrosoresorcin (394).
Diphenylamin Blue (479).
Diphenylamin orange (88).
Direct Gray (576).
Direct Violet (451).
Emerald Green (428).
Emerald Green cryst. (428).
English Brown (197).
Eosin (512).
Eosin A (512).
Eosin A ex. (512).
Eosin B (512).
Eosin bluish (517).
Eosin G G F (512).

Eosin J (512, 517).
Eosin 10 B (521).
Eosin 3J & 4J ext. (512).
Eosin Y (512).
Eosin Yellowish (512).
Erika B (78).
Erythrobenzin (448).
Erythrosin (517).
Erythrosin B (517).
Erythrosin D (517).
Erythrosin G (516).
Erythrosin yellow shade (517).
Ethyl Green (428).
Fast Blue (477).
Fast Blue B, spirit soluble (599).
Fast Blue R and 3R (601).
Fast Blue R, spirit soluble (599).
Fast Blue 2R, B, and 6B (601).
Fast Blue R, 2R, and 3R (639).
Fast Brown G (138).
Fast Green (427).
Fast Green J (428).
Fast Green O (394).
Fast Myrtle Green (394).
Fast Navy Blue R (639).
Fast Navy Blue RM and MM (639).
Fast Ponceau B (163).
Fast Red (102, 105).
Fast Red C (103).
Fast Red D (107).
Fast Red EB (107).
Fast Violet (620).
Fast Yellow (4, 8, 9, 88).
Fast Yellow extra (8).
Fast Yellow G (8).
Fast Yellow 053 (8).
Fast Yellow R (9).
Fine Blue (457).
Fuchsiacin (448).
Fuchsin (448).
Fuchsin cryst. (448).
Fuchsin S (462).
Gallocyanin DH and BS (620).
Gallocyanin RS, BS, and D (620).
Gentian Blue 6B (457).
Gentiana Violet (649).
Gentianin (649).
Gold Orange (86).
Gold Yellow (84).
Golden Yellow (2, 3).
Gray R and B (602).
Green E (428).
Green 087 (428).
Guinea Green (433).
Harmalin (448).
Helianthin (87).
Helvetia Blue (479).
Hessian Blue (457).
Hofmann Violet (450).
Hydrazin Yellow (94).
Imperial Scarlet (163).
Imperial Yellow (6).
Indigen D and F (599).
Indigo (689).
Indigo Carmine (692).
Indigo Carmine powd. IN (692).
Indigo extract (692).
Indigo pure BASF (689).
Indigotin (692).
Indigotin A (692).
Indisin (593).
Indisin R and B (601).
Indophenol (572).
Indophenol white (572).
Indulin opal. (599).
Indulin sol. (601).
Indulin, spirit soluble (599).
Indulin 3B opal. (599).
Indulin 6B opal. (599).
Indulin 3B sp. sol. (599).
Indulin 6B sp. sol. (599).
Indulin 3B (601).
Indulin 6B (601).
Iodeosin B (517).
Iodeosin G (516).
Iodin Green (459).
Iodin Violet (450).
Jaune Acide (4, 8).
Jaune Acide C (4).
Jaune Naphthol (3).
Jaune d'Or (3).
Jaune d'Orient (516).
Jaune Soleil (399).
Kaiser Yellow (6).
Kermesin Orange (97).
Leather Brown (197).
Leather Yellow (532).
Lemon Yellow (4).
Light Green S F bluish (434).
Light Green S F yellow shade (435).
London Blue extra (480)
Magdala Red (614).
Magenta (448).
Magenta F A B S Red (448).
Magenta Powder A (448).
Maize (399).
Malachite Green (427).
Malachite Green B (427–428).

Malta Gray (576).
Manchester Brown (197).
Manchester Brown EE (201).
Manchester Yellow (3).
Mandarin G R (97).
Mandarin G ext. (86).
Martius Yellow (3).
Mauve (593).
Mauve Dye (593).
Mauvein (593).
Meldola's Blue (639).
Metanil Yellow (95).
Methyl Blue water soluble (478).
Methyl Blue for silk MLB (478).
Methyl Blue O (479).
Methyl Violet (451).
Methyl Violet B (451).
Methyl Violet BB ext. (451).
Methyl Violet 3 BD (451).
Methylene Blue (650).
Methylene Blue A ext. (650).
Methylene Blue B and BG (650).
Methylene Blue B D (650).
Methylene Blue BB extra (650).
Methylene Blue BB extra D (650).
Methylene Gray (576).
Methylene Green G. conc. ext. (651).
Naphthalene Pink (614).
Naphthalene Red (614).
Naphthalene Rose (614).
Naphthol Black B (188).
Naphthol Black BDF (188).
Naphthol Blue R & D (639).
Naphthol Green (398).
Naphthol Green B (398).
Naphthol Orange (85).
Naphthol Red S (107).
Naphthol Yellow (3, 4).
Naphthol Yellow L (4).
Naphthol Yellow S (4, 5).
Naphthol Yellow SLOZ (4, 86).
Naphthylamin Yellow (3).
Naphthylene Yellow (2).
Navy Blue B (478).
New Coccin (106).
New Gray (576).
New Green (427).
New Methylene Gray (576).
New Red L (163).
New Victoria Green (427, 428).
New Yellow (88).
New Yellow L (8).
Nicholson Blue (477).
Night Green (459).
Nigrosin (576).
Nigrosin sol. (602).
Nigrosin sp. sol. (600).
Nitrodiphenylamin (6).
Nitromethylene Blue (651).
Oil Black (600).
Oil Orange 7078 (11).
Oil Yellow (16).
Opal Blue (457).
Orange (86).
Orange I (85).
Orange II (86).
Orange III (87).
Orange IV (88).
Orange A (86).
Orange A extra (86).
Orange Brown (17, 18, 41).
Orange A 1201 (86).
Orange B (85).
Orange extra (86).
Orange G (14).
Orange GG (14).
Orange GG Crystals (14).
Orange GRX (13).
Orange GS (88).
Orange GT (43).
Orange M (88).
Orange MN (95).
Orange N (43,88).
Orange O 27 (85).
Orange O (43).
Orange R (15, 55, 97).
Orange RN (43).
Orange RZ (85).
Orange T (97).
Orange 2 R (97).
Orange Y (86).
Orcellin No. 4 (102).
Paris Violet (451).
Phenylene Brown (197).
Philadelphia Yellow G (532).
Phloxin (521).
Phloxin T A (521).
Phosphin (532).
Picric Acid (1).
Pistachio (435).
Polychromin (659).
Pomona Green (459).
Ponceau B (163).
Ponceau 4 GB (13).
Ponceau 4 RB (160).
Ponceau G and GR (55).
Ponceau R (55).
Ponceau 3 RB (163).

Ponceau 2 G (15).
Ponceau 2 R (55).
Ponceau 6 RB (169).
Primula (450).
Primulin (659).
Printing Blue (599).
Pure Soluble Blue (480).
Pyoctanin Aureum (425).
Pyoctanin (451).
Pyrosin B (517).
Pyrosin J (516).
Quinolin Yellow, water-soluble (667).
Quinolin Yellow (667).
Rauracienne (102).
Red (107).
Red Violet 5 R extra (450).
Resorcin Yellow (84).
Resorcein O 275 (84).
Rhodamin (504).
Rhodamin B (504).
Rhodamin O (504).
Rhodamin B extra (504).
Rhodamin G and G extra (502).
Roccellin (102).
Rose Bengal (520).
Rose Bengal AT (520).
Rose Bengal G (520).
Rose Bengal N (520).
Rosein (448).
Rosolic acid (483).
Rubianite (448).
Rubidin (102).
Rubin (448).
Rubin S (462).
Russian Green (394).
Saffron Substitute (2).
Saffron Yellow (3, 4).
Safranilin (504).
Safranin (584).
Safranin AG, AGT, and OOF. (584).
Safranin Conc. (584).
Safranin extra G (584).
Safranin FF extra (584).
Safranin GOO. (584).
Safranin S. (584).
Safranin SP. (584).
Safranin T. (584).
Scarlet (55).
Scarlet L. (106).
Sloelin (600).
Sloelin RS. and BS. (601).
Solferino (448).
Solid Yellow (4).
Soluble Blue (480).
Spirit Black (600).
Spirit Blue (457).
Soluble Blue 8 B. (479).
Soluble Blue 10 B. (479).
Soluble Blue XL. (479).
Succinic (4).
Sudan I (11).
Sudan Red (614).
Sulphin (659).
Sulphin Yellow (4).
Sulphonaphthol Acid Yellow (4).
Sultan Red 4 B. (277).
Sun Yellow (399).
Tartrazin (94).
Thio Catechin (675).
Thio Catechin S. (675).
Toluidin Blue O. (654).
Tropæolin D. (87).
Tropæolin G. (95).
Tropæolin O (84).
Tropæolin OO (88).
Tropæolin OOO (85).
Tropæolin OOO No. 2 (86).
Tropæolin R (84).
Ursol D. (574).
Ursol DD. (574).
Ursol P. (574).
Vert Diamant (427).
Vert Lumière (459).
Vesuvin (197).
Vesuvin B. (201).
Victoria Blue B. (488).
Victoria Blue 4 R. (490).
Victoria Orange (2).
Victoria Yellow (2).
Victoria Yellow Conc. Z. (95).
Vidal Black (670).
Vidal Black S. (670).
Violein (593).
Violet de Methylanilin (451).
Violet R. (450).
Violet RR. (450).
Violet 5 R. (450).
Water Black (166).
Water Blue 6 B extra (480).
Water Blue (480).
Xanthin (532).
XL Soluble Blue (479).
Xylidin Red (55).
Xylidin Scarlet (55).
Yellow Corallin (483).
Yellow FY. (4).
Yellow MXX Conc. (95).
Yellow W (9).
Yellow WR. (89).

X. DOSAGE AND SYMPTOMS.

CONFECTIONERS' LIST AS A BASIS FOR A RULE.

Considering the Confectioners' List of 1899 as a correct guide as to which colors are harmful and which are harmless, the attempt has been made to determine how far dosage and the corresponding physiological effects may serve as a guide in determining which colors, other than those enumerated in either portion of the Confectioners' List, are harmful or harmless. (See p. 48.)

To this end the available literature has been searched and classified, and wherever it was possible to arrive at any conclusion as to the actual dose or the average dose over a stated period of time, and the corresponding physiological observations, these data have been separated and brought together for the purpose of making comparisons and deductions therefrom.

It was thought that the literature would show that if a dog or other animal is killed by a certain given amount of color per 100 pounds of body weight of the animal that such color is always harmful; that if untoward effects, such as vomiting, diarrhea, weakness, and general depression, are caused by more than a certain weight of color per 100 pounds body weight of the animal, such color is always regarded as harmless.

The classification of the available literature and the conclusions therefrom are as follows:

Of the 33 coal-tar colors listed as harmless in the Confectioners' List, 10, namely, G. T. 4, 9, 55, 65, 85, 103, 105, 107, 448, and 462, have been tested on humans, while the conclusion as to the remaining 23 is reached by the effects observed on dogs alone. However, contradictory statements are recorded in the case of No. 9, and none of these tests was of long-continued duration, but, on the contrary, in many cases the time covered was exceedingly short, and the conclusions deduced are, therefore, not necessarily final nor correct. It should be further noted that Nos. 95 and 106, reported as nonpoisonous to humans, are in the harmful section of this Confectioners' List.

I. Those colors which produced no effect are as follows (the number of grains given is the amount administered per 100 pounds body weight; where the data permitted, the number of days' duration of the experiment is also given:

		Grains.
5.	Brilliant Yellow S	532
13.	Ponceau 4 GB	113
521.	Phloxin	100
521.	Phloxin	300

II. Those colors which in some cases produced no effect, and in others produced effects, are as follows:

4. Naphthol Yellow S:
  395 grains produced diarrhea and no albuminuria.
  292 grains produced albuminuria.
  116 grains for 25 days produced no effect.
  25 grains for 2 weeks, on alternate days, produced no effect.
8 and 9. Fast Yellow Y and R:
  173 grains for 3 weeks produced no effect.
  53 grains produced albuminuria.
55. Ponceau 2 R.
  582 grains killed.
  198 grains produced no effect.
65. Fast Red B:
  143 grains for 145 days produced no effect.
  137 grains for 145 days produced no effect.
  98 grains for 145 days produced no effect.
  68 grains produced albuminuria.
  38 grains produced albuminuria.
103. Azorubin S:
  143 grains for 145 days produced no effect.
  137 grains for 145 days produced no effect.
  98 grains for 145 days produced no effect.
  70 grains produced diarrhea.
287. Azo Blue:
  233 grains produced no effect.
  166 grains produced albuminuria.

III. Those that produced only a slight disturbance which was regarded as negligible:

28. Archil Substitute V:
  301 grains produced vomiting and albuminuria.
  127 grains produced vomiting and albuminuria.
105. Fast Red E:
  70 grains produced diarrhea.
166. Wool Black:
  117 grains produced albuminuria.
240. Congo Red:
  192 grains produced albuminuria.
269. Chrysamin R:
  433 grains produced albuminuria.
  361 grains produced albuminuria.
394. Dinitrosoresorcin:
  139 grains produced loss of appetite.
  121 grains produced albuminuria.

From Class I above it would appear—

(*a*) That if a dose of 113 grains per 100 pounds body weight produced no effect it is to be classed as harmless.

From Class II above it would appear—

(*a*) That if albuminuria is caused by as little as 38 grains in some cases, and no effect is produced by doses as large as 143 grains, the color is to be classed as harmless.

(*b*) Also that if 198 grains produce no effect the color is to be classed as harmless, even if 582 grains in another case kill the animal.

(*c*) That if small amounts produce albuminuria and larger amounts do not the color is to be classed as harmless.

(*d*) That if small amounts produce diarrhea and larger amounts do not the color is to be classed as harmless.

From Class III it would appear that—

(*a*) Albuminuria produced by as little as 117 grains is to be regarded as not harmful.

(*b*) Diarrhea produced by 70 grains is to be regarded as not harmful.

(*c*) Vomiting and albuminuria produced by 127 grains are to be regarded as not harmful.

The colors classed as harmful in the Confectioners' List may be classified as follows:

I. *Dogs.*—Those colors that under certain conditions produce no noticeable effects on dogs, while under other conditions effects are observed, are classed as harmful colors:

1. Picric Acid:
    Dogs stand 5 grains daily for 18 days without effect.
    18½ grains produced diarrhea.
    20.4 grains killed.
2. Dinitrocresol:
    140 grains caused vomiting but did not kill.
    38½ grains caused vomiting but did not kill.
    38½ grains caused vomiting and did kill.
    35 grains caused vomiting but did not kill.
    31.6 grains caused vomiting but did not kill.
3. Martius Yellow:
    51 grains do not kill, but produce weakness, diarrhea, and albuminuria.
    50 grains kill.
    8 grains do not kill, but produce weakness, diarrhea, and albuminuria.

86. Orange II:
    714 grains produced kidney irritation, thirst, and diarrhea.
    333 grains kill, and produced diarrhea and albuminuria.
    244 grains produced kidney irritation, thirst, and diarrhea.
    36 grains produced no effect.

95. Metanil Yellow:
    620 grains produce vomiting.
    603 grains kill.
    407 grains no effect.
    371 grains kill.
    104 grains produced albuminuria.
    62 grains no effect.
    41 grains no effect.
    31 grains kill.

197. Bismarck Brown:
    246 grains produced vomiting.
    118 grains produced vomiting and general depression.
    38½ grains daily for 30 days, no effect.

398. Naphthol Green B:
    600 grains produced green urine and conjunctiva.
    292 grains produced no effect.
    128 grains produced green urine and conjunctiva.

II. Those colors that have not been observed to give negati results, but which have caused certain effects:

11. Sudan I:
    118 grains produced colored urine, vomiting, and albuminuria.
17 and 18. Chrysoidin Y and R:
    79 grains produced albuminuria.
    74 grains for 30 days produced no albuminuria, but a loss of 12½ per cent body weight.
88. Diphenylamin Orange:
    216 grains produced albuminuria.
    128 grains produced phenol in urine and albuminuria.
138. Fast Brown G:
    237 grains produced diarrhea and loss of appetite.
    218 grains produced diarrhea.

III. *Rabbits:*

1. Picric Acid:
    24.5 grains for 90 days, no effect.
    45 grains for 19 days kill.
86. Orange II:
    292 grains, no effect.
    933 grains kill.
95. Metanil Yellow:
    216 grains, no effect.

IV. *Humans:*

1. Picric Acid:
    8.3 grains no effect.
    13.8 grains no effect.
    Invalids and children can not stand this color.
2. Dinitrocresol:
    42 grains kill.
86. Orange II:
    1½ grains, no effect.
    3 grains, headache, vertigo, dryness of throat, and poor general condition.
95. Metanil Yellow:
    1½ grains, no effect.
    3 grains, no effect.

From Classes I and II above, it would seem to appear—

(*a*) That if a dog is killed the color is harmful, even though it take as much as 603 grains, or as little as 20.4 grains per 100 pounds body weight to kill.

(*b*) That if albuminuria is produced in dogs by as little as 79 grains, or as much as 128 grains, the color is harmful.

(*c*) That if diarrhea is produced in dogs by as much as 218 grains, or as little as 50 grains, the color is harmful.

(*d*) That even if a dog can take as much as 407 grains, the color may be harmful.

From Class III it would appear—

(*a*) That even if rabbits can stand as much as 292 grains, or as little as 24.5 grains, he color is harmful.

From Class IV it would appear—

(*a*) That if 42 grains kill a human, the color is harmful.

(*b*) That even if humans can stand as much as 3 grains without untoward effect, he color is harmful.

The conclusions that may be drawn from these data are:

1. If a dog is killed by—
   (*a*) 603 grains per 100 pounds body weight, the color may be harmful.
   (*b*) 582 grains per 100 pounds body weight, the color may be harmless.
2. If a dog can bear without effect—
   (*a*) 407 grains per 100 pounds body weight, the color may be harmful.
   (*b*) 198 grains per 100 pounds body weight, the color may be harmless.
3. If albuminuria is produced in a dog by—
   (*a*) 38 grains, or 143 grains per 100 pounds, body weight the color may be harmless.
   (*b*) 79 grains, or 128 grains per 100 pounds body weight, the color may be harmful.
4. If diarrhea is produced in a dog by—
   (*a*) 70 grains per 100 pounds body weight, the color may be harmless.
   (*b*) 50 grains per 100 pounds body weight, the color may be harmful.
5. If vomiting and albuminuria are produced in a dog by 127 grains per 100 pounds body weight, the color is not necessarily harmful.
6. If small amounts of color produce in a dog diarrhea or albuminuria, and larger amounts do not, the color may be harmless.
7. Even though rabbits can withstand 292 grains per 100 pounds body weight, the color is not necessarily harmless, but may be harmful.
8. If 42 grains kill a human, the color is harmful.
9. If a human can withstand 3 grains without effect the color is not necessarily harmless, but may be harmful.
10. If a human can not withstand 3 grains, even though it can withstand 1½ grains, the color is not necessarily harmless, but may be harmful.

Lehmann (*Methoden der praktischen Hygiene, 1890, p. 545*) says:

> I regard such substances as harmful to health which when fed to a sound dog in doses of a few decigrams per day produce at once, or after a few repetitions of the dose, disturbances in the health of the dog; on the other hand, dyes which in doses of from one to several grams can be taken for weeks on end without causing any disturbance or only slight intestinal disturbances or a slight and passing albuminuria can be regarded as harmless. It should never be forgotten that a few milligrams of a coal-tar color dyes very strongly and it is not easy—even by most extraordinary use of colored objects, e. g., by children—that more than milligrams, at most centigrams, of dye can be introduced into the human stomach. In extremely large doses many substances, for example, all our condiments, are naturally harmful.

Of 65 dogs weighed and experimented on by Chlopin the average weight was 9.2 kilograms, or 20.3 pounds; almost half the dogs (30) weighed between 6 and 11 kilos.

To adapt the rules of Lehmann to a basis of grains per 100 pounds of body weight, assuming the average weight of a dog to be 20 pounds, it is only necessary to multiply the weight of color by 5 and by 15.432. Doing so, the following data are obtained:

A. Harmful colors produce bad effects in doses of a "few" decigrams, i. e., multiples of a half gram; the word "few" is not sharply defined, but the rule means a "few" times 7.7 grains.

B. Harmless colors produce, when continuously fed, no bad effect in multiples of 10 of the doses of A, above, i. e., multiples of 77 grains.

C. Slight diarrhea and slight, temporary albuminuria are not to count against the color.

Applying these rules to the preceding colors discloses that they were apparently not so used, in selecting the harmless and harmful colors in the Confectioners' List.

LEHMANN'S RULES.

The Lehmann rules as just interpreted were applied to the data just given. As a result of such application the conclusion is reached that of the 15 colors classed as harmless (foregoing classes I, II, and III), 6 would be classed as doubtful by the Lehmann rules, namely:

13. Ponceau 4 GB,	65. Fast Red B,
8 and 9. Fast Yellow Y and R,	103. Azorubin S,
55. Ponceau 2R,	105. Fast Red E,

and the remaining 9 would have been classed as harmless.

Of the 11 classed as harmful (foregoing classes I, II, III, and IV), 2 would have been regarded as harmless, namely:

398. Naphthol Green B, 138. Fast Brown G,

3 would have been classed as doubtful, namely:

197. Bismarck Brown,	88. Diphenylamin Orange,
11. Sudan I,	

and the remaining 6 would have been classed as harmful.

SANTORI'S WORK AS A GUIDE TO A RULE.

Santori regards the following six dyes as harmless: 457 (188), 467 (74), 477 (129.5), 480 (156), 599 (64), 692 (467).

The first is the Green Table number and the bracketed figure the average number of grains per 100 pounds per day for 30 days.

In the case of Nos. 457 and 599 the animal was wholly normal throughout the test and the autopsy showed only normal conditions.

In the case of No. 477 the only disturbance was colored feces and the autopsy showed only normal conditions.

In the case of No. 480 the only disturbance was colored feces, but the autopsy showed a pea-green kidney.

In the case of Nos. 467 and 692 there was only vomiting; in the case of No. 467 the autopsy showed everything normal; whereas in the case of No. 692 the autopsy disclosed a swollen kidney.

Therefore, according to Santori, a dye that causes colored feces, even with colored kidney, is harmless. Also a dye that causes vomiting and swollen kidney is harmless. On the other hand, a dye (572) which produces no change or symptom observable during life, but fatty degeneration of the liver is shown at the autopsy, is a harmful dye. This lack of conformity makes the relation between dosage, symptoms, and harmfulness or harmlessness more confusing and perplexing.

YOUNG'S RULE.

It must be remembered that smaller amounts of drugs, and, therefore, of coal tar colors, effect children as a rule than are effective upon adults. Taking Young's rule as a guide, it appears that generally the effective dose for a 3-year-old child is one-fifth the effective adult dose; for a 4-year-old child one-fourth; for a 6-year-old child one-third; for an 8-year-old child two-fifths and for a 12-year-old child one-half the effective adult dose. All of this should be taken into account in drawing conclusions from experiments as to the harmlessness of any coal-tar dye upon humans. Very little attention has, however, been paid to this aspect of the matter in spite of the fact that colored foods, confectionery, pastry, beverages, and the like are partaken of by children and in many cases such articles are prepared for the sole or particular consumption of the very young.

From the foregoing data it seems clear that deductions as to the harmlessness or harmfulness of coal-tar dyes when administered to dogs, and not based upon autopsies, are not final nor conclusive as to the effect upon the dog. The extent to which such deductions are correctly transferable to humans is likewise not established.

XI. OIL-SOLUBLE OR FAT COLORS.

Oil-soluble colors are used for coloring fats, such as butter, oleomargarine, edible oils, and the like; of the coal-tar colors the oil-soluble colors are chemically nonsulphonated azo-colors.

The nonsulphonated azo-colors which have been physiologically examined are:

11. Sudan I (anilinazo-b-naphthol) (2).
16. Butter Yellow (anilin-azo-dimethylanilin).
17. Chrysoidin Y (anilin-azo-m-phenylene-diamin) (2).
18. Chrysoidin R (anilinazo-m-tolylene-diamin) (1).
41. Chrysoidin R (o-toluidin-azo-m-tolylene-diamin).
197. Bismarck Brown (m-phenylene-diamin-disazo-m-phenylene-diamin) (4).
201. Manchester Brown (m-tolylene-diamin-disazo-m-tolylene-diamin) (2).

The numbers preceding the trade names are the Green Table numbers; the scientific names appear in parentheses; and the number of dealers offering the colors on the United States market in the summer of 1907, out of a possible 12, appear after the scientific name, also in parentheses.

For all but No. 201 contradictory statements occur in the compiled literature; for No. 201 only unfavorable reports were found.

There were on the United States market in the summer of 1907 the following oil-soluble colors:

10. Sudan G (anilin-azo-resorcin) (1).
11. Sudan I (anilin-azo-b-naphthol) (2).
49. Sudan II (xylidin-azo-b-naphthol) (1).
60. Carminaph Garnet (a-naphthylamin-azo-b-naphthol) (1).

Of these No. 11 had been reported on contradictorily, and the remaining three had not been reported on at all. In addition to these the following three oil-soluble colors, not listed in the Green Tables and not reported on in literature, were wanted:

1. o-Toluidin-azo-b-naphthylamin (which does not seem to be described in literature in any way).
2. Amidoazo-toluol.
3. Anilin-azo-b-naphthylamin.

So that out of a total of seven oil-soluble colors on the United States market in the summer of 1907 only one had been examined physiologically and that with contradictory results. As before stated, these oil-soluble colors all belong to the class of nonsulphonated azo-colors.

Fraenkel (p. 575) says: "When, however, the azo-colors contain no sulpho-group (i. e., are nonsulphonated) they are poisonous. Thus for example, Bismarck Brown * * * Sudan I." Yet meta-nitrazotin, a nonsulphonated color not in the Green Tables, and probably not upon the market anywhere, and which is metanitranilin-azo-b-naphthol, is according to Weyl nonpoisonous.

Fraenkel (p. 575) also stated:

> The fact that the monazo colors examined by Cazeneuve and Lépine are harmless, as above stated, is equally explained by the constitution of these substances. Those two investigators examined (omitting the trade names) a-naphthylamin-sulphoacid-azo-a-naphthol-a-sulphoacid, a-naphthylamin-sulphoacid-azo-b-naphthol-disulphoacid, a-naphthylamin-azo-b-naphthol-disulphoacid, xylidin-azo-b-naphthol-disulphoacid, sulphanilicacid-azo-a-naphthol, Amido-azo-toluene-disulphonicacid.
>
> These substances are all sulphoacids and the sulpho-groups here effect the depoisoning of the original substance.

Examining this statement it therefore appears that in Fraenkel's opinion, at least, a-naphthylamin-azo-a-naphthol, a-naphthylamin-azo-b-naphthol, xylidin-azo-b-naphthol, anilin-azo-a-naphthol, and amido-azo-toluol are in and of themselves poisonous substances, and are rendered nonpoisonous by sulphonation. Diligent search through the literature has failed to uncover any original communications of investigators to that effect.

With but one known exception all the nonsulphonated azo-colors are regarded by Fraenkel as poisonous. The colors 17, 18, 41, 197, and 201 on page 159 are not used as oil-soluble colors and therefore form no comparative basis for judging oil-soluble colors except that both are nonsulphonated azo-colors; in Fraenkel's opinion, however, such a comparison is wholly justified and proper.

Excluding now 17, 18, 41, 197, and 201 for the purposes of comparison, the following tabulation is made:

Oil-soluble nonsulphonated azo-colors on the market, 1907, deemed poisonous by Fraenkel.

Oil-soluble colors.	On United States market.	Fraenkel calls poisonous.
anilin-azo-resorcin	x	
anilin-azo-b-naphthol	x	x
xylidin-azo-b-naphthol	x	x
a-naphthylamin-azo-b-naphthol	x	x
o-toluidin-azo-b-naphthylamin	x	
amido-azo-toluol	x	x
anilin-azo-b-naphthylamin	x	
a-naphthylamin-azo-a-naphthol		x
anilin-azo-a-naphthol		x

That is, the only ones on the United States market concerning which no expression of specific opinion is to be found in Fraenkel are the following three: anilin-azo-resorcin, toluidin-azo-b-naphthylamin, and anilin-azo-b-naphthylamin, and nothing is published on the physiological action of any of them specifically. The probability, however, would seem to be that they are not harmless, and until their harmlessness is positively established their exclusion from the permitted list of coal-tar colors for use in foods seems to be the only safe and proper course to be followed. The physiological action of so-called "Butter Yellow" No. 16 of the Green Tables (see p. 85), merely strengthens this conclusion.

XII. RULES AND REASONS FOR SELECTING THE SEVEN COLORS PERMITTED BY F. I. D. 76.

STATEMENT OF RULES.

In view of the confusion and uncertainty disclosed in the foregoing literature relative to the physiological action of coal-tar colors, the difficulties in the way of making a list of coal-tar colors to be permitted in the coloring of food products are seen to be by no means slight. The following rules governing selection were in mind during the making of the list given in Food Inspection Decision No. 76, and the closeness with which they were followed is discussed on page 166.

Rule I. All colors which have not been physiologically tested either upon animals or man shall not be permitted for use in foods.

Rule II. All coal-tar colors which have been examined physiologically with contradictory results shall not be permitted for use in foods.

Rule III. All coal-tar colors which have been examined physiologically and have been declared to be of doubtful harmlessness shall not be permitted for use in foods.

Rule IV. Only those coal-tar colors whose chemical composition was definitely disclosed or otherwise ascertained, and which were on the United States market in the summer of 1907, and which have been examined physiologically and with no other than a favorable result shall, for the present, be permitted for use in foods.

ANALYSIS OF THREE RECOMMENDATIONS MADE TO THE DEPARTMENT OF AGRICULTURE.

These rules were formulated as a guide in view of the divergent opinions expressed in three different recommendations to the Department of Agriculture. One of these recommendations suggested that permitted colors be designated by nine titles. Comparison of these titles with the Green Tables and with the tabulated survey of the unfavorable, favorable, and contradictory literature corresponding to the Green Table numbers (p. 63) discloses the following facts:

Comparison of nine suggested color titles with corresponding Green Table numbers and the reports on the same.

Name.	Green Table No.	Unfavorable.	Favorable.	Contradictory.	No report.[1]
Chrysoidin	17			x	
	18			x	
	41			x	
Tropæolin	84			x	
	85		x		
	86			x	
	87			x	
	88			x	
	95			x	
Azoflavin	92		x		
Roccellin	102		x		
Ponceau	13			x	
	15			x	
	44				x
	55			x	
	56				x
	57				x
	108				x
	114				x
	146				x
Ponceau	147				x
	148				x
	150				x
	160			x	
	163			x	
	169		x		
Bordeaux	65		x		
	107		x		
	157				x
	170				x
	171				x
	198				x
	244				x
Biebrich Red (?)	20				x
Sulphonated Fuchsin	402		x		
Naphthol Yellow S	4		x		
Total	36	0	8	13	15

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[1] Of physiological tests in literature compiled.

Therefore, under 9 titles 36 different chemical individuals would be placed upon the permitted list, of which only 8, or less than 25 per cent, have been examined physiologically with only favorable results, and 28, or more than 75 per cent, had either not been examined at all physiologically or with contradictory results.

The 8 of these chemical individuals examined with only favorable results, and the number of sources out of a possible 12 offering them on the United States market in the summer of 1907, are as follows:

Colors reported on favorably and number of dealers handling same.

Green Table No.	Sources offering.	Green Table No.	Sources offering.
[1]*4*	10	102	0
65	2	*107*	7
85	2	169	1
92	0	462	2

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

Another recommendation suggested the permissive use of 42 entries in the Green Tables, which are tabulated below in the same manner as the suggestions in the preceding recommendation:

Comparison of 42 recommended Green Table numbers with reports in the literature.

Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]	Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]
4		x			427			x	
8			x		434	x			
17			x		448			x	
50				x	451			x	
53				x	456				x
54				x	457			x	
55			x		462		x		
65		x			477		x		
85		x			478	x			
86			x		479	x			
89		x			480			x	
92		x			504			x	
94	x				512		x		
102		x			513				x
103		x			514				x
107		x			516	x			
138			x		*517*		x		
163			x		518				x
166		x			562				x
240		x			601			x	
269			x						
287			x		Total	5	14	15	8

[1] Of physiological tests in compiled literature.

Applying the conclusions hereinbefore reached to these entries, it is found that 14 out of the 42 colors recommended, or exactly one-third, had been examined physiologically with only favorable results, and the remaining two-thirds had been either examined physiologically with only unfavorable or with conflicting results, or had not been examined at all.

The 14 chemical individuals examined with favorable result and the number of sources out of a possible 12 offering them on the United States market in the summer of 1907 are as follows:

Colors reported on favorably and number of dealers handling same.

Green table No.	Sources offering.	Green table No.	Sources offering.
[1]*4*	10	*107*	7
65	2	166	0
85	2	240	1
89	1	462	2
92	0	477	0
102	0	512	3
103	6	577	5

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

The third of these recommendations suggested 27 different chemical individuals for permissive use; these are tabulated below in the same manner as the suggestions in the preceding recommendations.

Twenty-seven colors recommended for use and reports in the literature on the same.

Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]	Green table No.	Un-favor-able.	Favor-able.	Equiv-ocal.	No report.[1]
4	....	x	....	....	481	....	....	....	x
16	....	....	x	....	512	....	x	....	....
17	....	....	x	....	516	x	....	....	....
18	....	....	x	....	517	....	x	....	....
41	....	....	x	....	518	....	....	....	x
65	....	x	....	....	521	....	x	....	....
85	....	x	....	....	532	x	....	....	....
102	....	x	....	....	584	....	....	x	....
197	....	....	x	....	601	....	....	x	....
201	x	....	....	....	602	x	....	....	....
398	....	....	x	....	650	....	....	x	....
457	....	....	x	....	*692*	....	x	....	....
462	....	x	....	....					
476	....	....	....	x	Total	4	10	10	3
477	....	x	....	....					

[1] Of physiological tests in literature compiled.

Therefore, applying the same method of drawing conclusions as in the case of the preceding two recommendations, it appears that 10 out of the 27 suggested chemical individuals had been examined physiologically with only favorable results, and the remaining 17 had either not been examined at all or with unfavorable or conflicting results. The 10 chemical individuals examined with favorable results, and the number of sources out of a possible 12 offering them on the United States market in the summer of 1907, are as follows:

The 10 recommended colors favorably reported on in the literature and the dealers handling same.

Green Table No.	Sources han-dling.	Green Table No.	Sources han-dling.
[1]*4*	10	477	0
65	2	512	3
85	2	*517*	5
102	0	521	0
462	2	*692*	3

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

All of the colors included in these three recommendations concerning which only favorable reports were found in the literature are given in the following table:

Recommended colors favorably reported on in the literature.

Green Table No.	Recommendations.				Dealers offering.	Green Table No.	Recommendations.				Dealers offering.
	I.	II.	III.	Total.			I.	II.	III.	Total.	
[1] *4*	x	x	x	3	10	240	......	x	......	1	1
65	x	x	x	3	2	462	x	x	x	3	2
85	x	x	x	3	2	477	......	x	x	2	0
89	......	x	......	1	1	512	......	x	x	2	3
92	x	x	......	2	0	*517*	......	x	x	2	5
102	x	x	x	3	0	521	......	......	x	1	0
103	......	x	......	1	6	*692*	......	......	x	1	3
[1] *107*	x	x	......	2	7	Total .	8	14	10	......	....
166	......	x	......	1	0						
169	x	......	......	1	1						

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

This table is now rearranged to show—

I. *The recommended colors not offered in the United States market in the summer of 1907.*

Green Table No.	Recommended by—	Green Table No.	Recommended by—
92	2	477	2
102	3	521	1
166	1		

II. *The recommended colors offered on the United States market in the summer of 1907.*

Green Table No.	Recommended by—	Sources handling.	Green Table No.	Recommended by—	Sources handling.
[1] *4*	3	10	169	1	1
65	3	2	240	1	1
85	3	2	462	3	2
89	1	1	512	2	3
103	1	6	*517*	2	5
107	2	7	*692*	1	3

[1] The italicized Green Table numbers are those of the permitted list of Food Inspection Decision No. 76.

It appears that out of 17 different chemical individuals suggested by these 3 recommendations jointly, 5 were not on the United States market in the summer of 1907, and 12 were on that market; also that of these 12, 4 were suggested by all 3 recommendations; 3 were wanted by only 2 out of the 3, and the remaining 5 were spoken for by only one. Examining all of the recommendations, it appears that out of a total of 79 recommendations 17 had been examined with only favorable results, and that the remaining 62 were either not examined or had been examined with unfavorable or conflicting results.

To add to the confusion the third recommendation mentioned suggested that the following Green Table numbers should not be permitted for use in food products because they are harmful: 1; 2; 3; 6; 86; 95; 425; 451; 454; 480; 483; 484; 487; 488; 490; 571; 584; 599; 620; 624; 650. The same paper, however, recommended that Nos. 584 and 650 be permitted for use in food products; that No. 86, suggested by both the other recommendations, be prohibited; and that Nos. 95, 451, and 480, suggested by one or the other of the remaining two recommendations, be likewise prohibited for use in food products. It must be clear to everyone that these three recommendations are not of one mind as to (1) the colors which are harmless; (2) the colors which are harmful; (3) the colors which are used or are useful in food coloring; and (4) the colors which should be used in food coloring. Such a state of affairs fully justifies rules of the scope and intent of those formulated at the beginning of this section for making a selection of permissible food colors.

PROCESS OF ELIMINATION.

From the foregoing data it appears that out of 80 different chemical individuals on the food-color market of the United States in 1907, only 16 had been examined physiologically with a favorable result (see p. 64).

These 16, arranged according to the shade produced, are as follows (numbers preceding the names are the Green Table numbers; the numbers in parentheses show the number of sources desiring the color; the italicized Green Table numbers are the ones finally selected by Food Inspection Decision No. 76):

REDS.

65. Fast Red B (Alphanaphthylamin azo-R-salt) (2).
103. Azorubin S (Naphthionic acid azo NW acid) (6).
105. Fast Red E (Naphthionic acid azo-Schaeffer acid) (1).
107. Amaranth (Naphthionic acid azo-R-salt) (7).
169. Crocein Scarlet 7B (Amidoazotolutol-monosulphonic-acid-azo-betanaphthol-monosulphonic acid B) (1).
240. Congo Red (Benzidin disazo-naphthionic acid) (1).
462. Acid Magenta (Magenta trisulphonic acid) (2).
512. Eosin A (Tetra-bromo-fluorescein) (3).
517. Erythrosin (Tetra-iodo-fluorescein) (5).
520. Rose Bengal (Dichlor-tetraiodo-fluorescein) (2).

ORANGE.

85. Orange I (Sulphanilic acid azo-alpha-naphthol) (2).

YELLOWS.

4. Naphthol Yellow (Dinitroalphanaphtholsulpho acid) (10).
89. Brilliant Yellow S (Sulphanilic acid azo-diphenylamin, sulphonated) (1).

GREENS.

433. Guinea Green B (Benzyldehyde + benzylethyanilin sulpho-acid) (1).
435. Light Green S F yellowish (Benzyldehyde+benzylethylanilin+sulphonation) (4).

BLUE.

692. Indigo Carmine (Indigo disulpho acid) (3).

Considering now the reds, and particularly 107, which, as the number appearing in parentheses after the scientific name indicates, was wanted by 7 sources out of the 12 drawn on, this being the most desired of all the reds, it would seem reasonable to believe that all the wants supplied by 65, 103, 105, 169, 240, and 462 would be covered by 107. Chemically 107 is closely allied to 65, 103, 105, and tinctorially it is likewise closely allied to 169, 240, and 462.

Nos. 512, 517, and 520 are chemically quite different from the other members of this group, and tinctorially they differ, being of a particularly brilliant shade, and tinctorially more powerful; 517 being desired by 5 out of the 12 sources drawn upon, and thus being the most desired of these three colors, was selected in the expectation that any work 512 and 520 could do in food products would be equally well done by 517. For reds, therefore, the choice fell upon 107 and 517.

Orange.—Only one color was wanted, and that by 2 out of the 12 sources, and this was placed in the permitted list.

Yellows.—The choice of No. 4, which was wanted by 10 out of the 12 sources, was made in the expectation that every purpose that 89 could serve in food products could be served also by 4.

Greens.—Tinctorially and chemically the two greens are very closely allied, and in view of the reasonableness of the expectation that 435 could do all the work of 433 as well, its choice was regarded as justified, especially as 435 was wanted by 4 out of the 12 sources, as against only one for 433.

Blue.—No. 692 being regarded as harmless by all, and being the only blue in the list, it was selected.

In this manner six out of the seven permitted colors were selected.

REASONS FOR ADDING PONCEAU 3R.

An examination of the table on page 20 discloses the fact that among the reds desired four were azo-reds made from anilin derivatives as the first component, namely:

53. Xylidin-azo-alphanaphtholdisulpho acid.
54. Xylidin-azo-Schaeffer acid.
55. Xylidin-azo-R-salt.
56. Cumidin azo-R-salt.

Each of these was wanted by 1 source out of the possible 12. This was construed as an expression of a real need in the art of food coloring for a color of this class. The reason for such a need was not then, nor is it now, apparent, but the propriety of giving even a seeming need due consideration was regarded as justified. For only one of the four desired, namely, 55, could any specific references in the literature be found, and, in fact, four references were found favorable thereto and three unfavorable thereto; thus eliminating 55 from consideration, leaving 53, 54, and 56.

The Austrian law of January 22, 1896 (see Lieber, *p. 15*), permits the use of azo colors derived from higher homologues of anilins, beginning with xylidin and sulphonated betanaphthols. As Nos. 53 and 54 were both derived from xylidin, it was regarded as safe to assume that they would probably be as objectionable as 55. This left 56 only to be considered, which color is derived from cumidin and R-salt. The cumidin portion satisfies the Austrian law and also satisfies the general law laid down in Fraenkel (*p. 162*), namely, that the greater the number of ring-methyls the less the toxic property of the resulting compound, because of the oxidation of these methyls to carboxyls in the animal system; the R-salt portion satisfies the general law laid down in Fraenkel, that the more highly sulphonated the less toxic a substance becomes, and therefore 56 is in the first portion of its composition of such a nature as to be less objectionable than 55, if 55 be objectionable.

This combination of facts, namely, the desirability of an anilin azo-red, the provision for diminishing the toxicity of 55 by the substitution of cumidin for xylidin therein, and the general provision in the Austrian food law, made it appear desirable and safe to take into account an anilin azo-red, and therefore to select 56 as probably the least objectionable, if it be at all objectionable, of the anilin azo-reds.

The full list of permitted colors was therefore extended to 7, as follows:

Red shades.—107. Amaranth; 56. Ponceau 3R; 517. Erythrosin.
Orange shade.—85. Orange I.
Yellow shade.—4. Naphthol Yellow S.
Green shade.—435. Light Green S F yellowish.
Blue shade.—692. Indigo disulpho acid.

It should be noted that with respect to tetra-iodo-fluorescein, 517 of the Green Tables, no specific investigation, pronouncing it harmless or harmful, is described in the literature. No. 516, the diodo-fluorescein, is reported specifically adversely in the literature, especially by Chlopin; the Confectioners' List, the laws of Austria, the laws of France, the rather superficial examination of No. 517 by Grandhomme, and the statement in Fraenkel (*p. 574*), that it produces no disturbance, are all taken to apply to 517 and not to 516. The identification of the color under examination with 517 has not in all cases been satisfactorily exclusive, but it is believed that the differentiation of the harmful 516 by Chlopin makes all the other references cited pertinent to 517.

With respect to 435, it must be pointed out that 434, which is the methyl instead of the ethyl derivative, has only been regarded as suspicious, and one examination of 435, namely, that of Lieber (*p. 144*), does not appear to disclose anything which would positively exclude 435.

QUALITY, CLEANLINESS, AND EFFICIENCY.

The justification for limiting the permitted colors to 6 out of 16, against which nothing unfavorable is contained in the literature and regarding which favorable statements are at hand, and the addition to these 6 of the seventh color is to be found in the very great variation in tinctorial quality, in percentage of coloring matter, in amounts of insoluble matter, both organic and inorganic, and in the amounts of organic matter not coloring matter, as well as in the large number of samples containing an amount of arsenic in excess of that permitted by the United States Pharmacopœia for the only coal-tar color therein mentioned, namely, Methylene Blue, and the varying amounts of heavy metals, such as copper, lead, and iron, mostly in excess of the limits permitted in the Pharmacopœia for various medicinal chemicals. This wide variation in quality and degree of cleanliness, all pointing to a very great difference in the care with which coal-tar colors offered for food purposes are prepared, render the conclusion safe that some control over food colors in respect to quality is desirable, necessary, and essential.

The efficiency of the 7 colors, 6 of which were selected from among the 16 considered for the purpose of making this list of permitted colors as being reasonably sure to be harmless, is evidenced by the fact that, although the addition of colors has been sought by persons interested in the food-coloring art, not one of the remaining 10 colors of those 16 has been so requested. In other words, the colors that the department has been requested to add to the permitted list were outside of the 16 colors which were on the markets of the United States in 1907, and were described in the literature in such a manner as to lead to the conclusion that they were probably not harmful.

XIII. LISTS OF COLORS SUBSEQUENTLY RECOMMENDED BY INDIVIDUALS AND ASSOCIATIONS.

Since Food Inspection Decisions Nos. 76 and 77 were published recommendations of lists of permitted colors have been made by other individuals and by a voluntary association. For the purpose of comparing these proposed lists of permissible colors with the permitted list of Food Inspection Decision No. 76, the former are now to be examined in the same manner as the coal-tar colors, on the United States market in the summer of 1907 for food coloring purposes, were examined in order to establish the permitted list of 7 colors. These recommendations cover the following lists:

1. W. Ernst's list.
2. Muttelet's interpretation of the French law.
3. Second International White Cross Congress list.
4. Beythien and Hempel's list.
5. An American manufacturer's list.
6. Béhal's list.

ERNST.

W. Ernst (*Färber Zeitung 1908, vol. 19, p. 381;* abst. *Chem. Ztg.* "*Reportorium*" *1909, p. 89*) recommends for use in foods the following 38 titles of coal-tar colors. The Green Table numbers appear in parentheses after each title where corresponding numbers could be ascertained; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.

1. Auramin (425, 426).
2. Naphthol Yellow S (*4*).
3. Quinolin Yellow (666, 667).
4. Tartrazin (94).
5. Acid Yellow (8, 88, 95, *4*).
6. Spirit Yellow (Amidoazobenzol) (7).
7. Curcumin S (399).
8. Eosin (512, 514, 515, *517*, 521).
9. Erythrosin (516, *517*).
10. Fluorescein (510).
11. Orange II (86).
12. Crocein Orange (13).
13. Basic Oranges (?).
14. Fast Red (63, 65, 102, 103, 105, *107*, 144).
15. Amara Red (?).
16. Naphthol Red (?).
17. Azo red (62).
18. Bordeaux (65, *107*, 157, 170, 171, 198, 244).
19. Victoria Rubin (?).
20. Ponceau (13, 15, 44, 55, *56*, 57, 108, 114, 146, 147, 148, 150, 160, 163, 169, 448).
21. Rhodamin (496, 497, 498, 502, 504, 505).
22. Acid Magenta (462).
23. Safranin (583, 584, 585).
24. The Croceins (13, 104, 106, 145, 151, 160, 164, 169).
25. Acid Green (434, *435*).
26. Brilliant Fulling Green (?).
27. Malachite Green (427, 428).
28. Brilliant Green (428).
29. Water Blue (480).
30. Patent Blue (440, 442).
31. Brilliant Fulling Blue (?).
32. Domingo Blue B extra (?).
33. Methyl Violet (451, 454).
34. Acid Violet (464, 465, 467, 468, 470, 471, 472, 474, 507).
35. Bismarck Brown (197, 201).
36. Acid Brown (133, 138).
37. Nigrosin (600,602).
38. Several Acid Blacks (184 and ?).

Of these 38 titles only 11 refer to a single entry each in the Green Tables (namely 2, 4, 6, 7, 10, 11, 12, 17, 22, 28, 29); 7 titles can not be definitely connected with any entry in the Green Tables (titles 13 15, 16, 19, 26, 31, 32); one title is broader than the corresponding color in the Green Tables (title 38) and the remaining 19 titles each and all refer to more than one entry in the Green Tables.

These 31 titles embrace 88 different entries in the Green Tables; some of these entries are included in two or more titles as follows:

Green Table number.	Titles.
4	2, 5
13	12, 20, 24
65	14, 18
107	14, 18
160	20, 24
169	20, 24
428	27, 28
517	8, 9

These 88 different Green Table entries can be divided as follows on the basis of the compilation of literature on physiological action hereinbefore given (see p. 63):

Unfavorable only.—94, 164, 201, 425, 434, 502, 516, 602, 667. Total 9, or 10.2 per cent.

Favorable only.—*4*, 65, 102, 103, 105, *107*, 169, 399, *435*, 462, 467, 512, *517*, 521, 600. Total 15, or 17.0 per cent.

Conflicting.—8, 13, 15, 55, 86, 88, 95, 106, 138, 160, 163, 197, 427, 428, 448, 451, 480, 504, 584. Total 19, or 21.6 per cent.

Not reported on.—7, 44, 56[1], 57, 62, 63, 104, 108, 114, 133, 144, 145, 146, 147, 148, 150, 151, 157, 170, 171, 184, 198, 244, 426, 440, 442, 454, 464, 465, 468, 470, 471, 472, 474, 496, 497, 498, 505, 507, 510, 514, 515, 583, 585, 666. Total 45, or 51 per cent.

According to this mode of judging only 15, or 17 per cent, of the colors suggested by Ernst for food coloring would be regarded as proper for use in foods.

These 15 embrace 4 of the 7 permitted colors of Food Inspection Decision No. 76, namely, 4, 107, 435, and 517, leaving 11 to be considered. Of these, 5 were not on the United States market in the summer of 1907, namely, 102, 399, 467, 521, and 600.

The remaining 6 are as follows (the numbers in parentheses being the number of sources out of a possible 12 offering them on the United States market in the summer of 1907): 65 (2); 103 (6); 105 (1); 169 (1); 462 (2); and 512 (3).

The reasons for the noninclusion of these in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

MUTTELET'S INTERPRETATION OF THE FRENCH LAW.

Muttelet (*Annales des Falsifications, 1909, pp. 26–38*), places the following interpretation on the French regulations of December 29, 1890, and of August 4, 1908, classifying them as—

I. Those colors which are certainly permitted by those regulations.
II. Those colors whose permitted or prohibited use is doubtful.
III. Those colors which are certainly prohibited.

The Green Table numbers contained in each class are classified as follows (the italicized numbers being those of the permitted list of Food Inspection Decision No. 76): (*a*) Unfavorably reported; (*b*) favorably reported; (*c*) conflictingly reported; (*d*) not reported in the literature as to their physiological action (see p. 63); in parentheses is given the number of sources out of a possible 12 offering those colors on the United States market in the summer of 1907.

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[1] See page 167 for special reasons for including Ponceau 3R in permitted list.

I. Those colors which are certainly permitted:

(*a*) None. (*b*) *4* (10); 5 (0); 462 (2); 512 (3); *517* (5); 520 (2); 600 (0); total, 7. (*c*) 55 (2); 427 (2); 451 (5); 457 (0); total, 4. (*d*) 158 (0); 518 (2); total, 2.

II. Those colors whose permitted or prohibited use is doubtful:

(*a*) None. (*b*) 65 (2); *107* (7); 599 (0); total, 3. (*c*) 8 (5); 84 (2); 427 (2); 457 (0); 601 (1); total, 4. (*d*) 452 (2); 456 (0); 513 (0); 514 (0); total, 4.

III. Those colors which are certainly prohibited:

(*a*) 3 (0); (*b*) none; (*c*) 427 (2); (*d*) none.

It will be noted that 427 appears in all three of Muttelet's classifications. The reason for this is that 427 is or has been marketed in at least three different forms; of these the straight chlorhydrate is permitted, the oxalate and the zinc chlorid double salt are of doubtful admissibility, and the picrate is undoubtedly forbidden.

Also 457 appears in Muttelet's Classes I and II because the "Bleu Lumière" of Muttelet's Class I is indistinguishable from his "Bleu Lumière" of Class II, when using the Green Tables as a guide. Further, this list of Muttelet contains only 3 out of the 7 colors permitted in Food Inspection Decision No. 76, namely, 4 and 517 of his Class I and 107 of his Class II.

An inspection of Muttelet's Class I discloses 13 Green Table entries, of which only 7 have been reported in the literature, as herein compiled and rated, in a favorable manner; of these 7, 2 were not on the United States market in the summer of 1907 and of the remaining 5, 2 are in the permitted list of Food Inspection Decision No. 76; the remaining 3 are: 462. Acid Magenta (2); 512. Eosin (3); 520. Rose Bengal (2), and the reasons for whose noninclusion in the permitted list of Food Inspection Decision No. 76 have been given (p. 167).

SECOND INTERNATIONAL WHITE CROSS CONGRESS.

The Second International White Cross Congress, held in Paris, October 18 to 24, 1909, according to the Chemiker Zeitung, 1909, page 1227, adopted the following list of colors which were said to be proper for use in coloring food products. The figures in parentheses are the Green Table numbers; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.

1. Erythrosin (516, *517*).
2. Rhodamin B (504).
3. Bordeaux S (*107*).
4. Bordeaux G (170).
5. Fast Red E (105).
6. New Coccin (106).
7. Ponceau 2R (55).
8. Xylidin Scarlet (55).

9. Magenta (448).
10. Acid Magenta (462).
11. Orange I (*85*).
12. Naphthol Yellow S (*4*).
13. Chrysoin (84).
14. Auramin O (425).
15. Acid Green (434, *435*).
16. Lyons Blue (457).
17. Patent Blue (440, 442).
18. Paris Violet (451).
19. Acid Violet (464, 465, 467, 468, 470, 471, 472, 474, 507).
20. Black Indulins (599).
21. Sulphonated Nigrosin (602).

It will be observed that titles 7 and 8 refer to the same Green Table number; this leaves, therefore, only 20 titles to consider. These refer to 31 different Green Table numbers which are classified as (*a*) only unfavorable reports, (*b*) only favorable reports, (*c*) conflicting reports, and (*d*) no reports, in the literature hereinbefore compiled and rated (see p. 63):

(*a*) 425, 434, 516, 602; total, 4.
(*b*) *4*, *85*, 105, *107*, *435*, 462, 467, *517*, 599; total, 9.
(*c*) 55, 84, 106, 448, 451, 457, 504; total, 7.
(*d*) 170, 440, 442, 464, 465, 468, 470, 471, 472, 474, 507; total, 11.

The numbers of section (*b*), of which only favorable reports are recorded, are the only ones here of interest; they are 9 in number, or less than 30 per cent of all those included in this list, and of these 9, 5 are on the permitted list of Food Inspection Decision No. 76. The remaining 4 are the following, the number in parentheses representing the number of sources out of a possible 12 offering them on the United States market in the summer of 1907:

105. Fast Red E (1).
462. Acid Magenta (2).
467. Acid Violet 6 B, not offered.
599. Printing Blue, not offered.

The reasons for the noninclusion of these colors in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

BEYTHIEN AND HEMPEL.

Beythien and Hempel (*Färber Ztg., 1909, v. 15, pp. 301, 348, 392, 436;* abst. *Chem. Ztg., 1910, p. 58*) recommend the following colors for use in food products. (The numbers in parentheses are the corresponding Green Table numbers, where such connection could be established; the italicized numbers are those of the permitted list of Food Inspection Decision No. 76.)

1. Alizarinblue (562, 563).
2. Amaranth (*107*).
3. Bordeaux Red (?).
4. Brilliant Blue (?).
5. Diamondfuchsin (448).
6. Fast Blue (477, 599, 601, 639, 640).
7. Fast Yellow R (9).
8. Fast Red (63, 65, 102, 103, 105, *107*, 144).
9. Eosin (512, 514, 515, *517*, 521).
10. Erythrosin (516, *517*).
11. Fuchsin S (462).
12. Light Green S F yellowish (*435*).
13. Indigo disulphoacid (*692*).
14. Indulins (599, 601, 603).
15. Light Blue (?).
16. Malachite Green (427, 428).
17. Methyl Violet (451, 454).

18. Naphthol Yellow S (*4*).
19. Orange I (*85*).
20. Orange L (54).
21. Paris Violet (451).
22. Phloxin (518, 521).
23. Ponceau 3R (*56*, 57).
24. Ponceau Red (?).
25. Primrose (3, 513, 514).
26. Roccellin (102).
27. Roscellin (?).
28. Rubin (448).
29. Acid Yellow S (*4*).
30. Acid Magenta (462).
31. Solid Blue (?).
32. Tropaeolin OOO (*85*, 86).
33. Waterblue (480).

For six titles (3, 4, 15, 24, 27, and 31) no corresponding Green Table number could be determined.

Of the remaining 27 titles, No. 9 includes part of No. 10, 32 includes 19, 9 includes part of 25, 8 includes 26, 17 includes 21, 11 is identical with 30, and 18 is identical with 29, all on the assumption that the correct connections between title and Green Table numbers have been made. Therefore these 27 titles are, in fact, only 25 titles; of these 25 titles, 13 refer to but one Green Table number each; the remaining 12 titles each refer to two or more Green Table numbers.

The Green Table numbers above given are now arranged in the following four classes according to the literature hereinbefore compiled and rated (see p. 63):

(*a*) Only unfavorable reports: 3, 516, 639; total, 3.

(*b*) Only favorable reports: *4*, 65, *85*, 102, 103, 105, *107*, *435*, 462, 477, 512, *517*, 521, 599, *692*; total, 15.

(*c*) Conflicting reports: 9, 86, 427, 428, 448, 451, 480, 563, 601; total, 9.

(*d*) No reports: 54, *56*,[1] 57, 63, 144, 454, 513, 514, 515, 518, 562, 603, 640; total, 13.

The 15 Green Table numbers of class (*b*) are the only ones here of interest; they include 6 out of the 7 permitted colors of Food Inspection Decision 76; of the remaining 9, 4 (102, 477, 521, 599) were not on the United States market in the summer of 1907. The remaining 5 are as follows. (The number in parentheses is the number of sources out of a possible 12 offering them on the United States market in the summer of 1907):

65. Fast Red B (2).
103. Azorubin S (6).
105. Fast Red E (1).
462. Acid Magenta (2).
512. Eosin (3).

The reasons for the noninclusion of these colors in the permitted list of Food Inspection Decision No. 76 have been given on page 167.

SUMMARY OF THREE PRECEDING RECOMMENDATIONS.

The recommendations made by Ernst, the White Cross Congress, and Beythien and Hempel are summarized in the following table:

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[1] See page 167 for special reasons for permitting use of Ponceau 3R.

Summary of recommendations from three sources.

Green Table No. (11).	Unfavorable reports only.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
3			x	1	0
94	x			1	6
164	x			1	0
201	x			1	2
425	x	x		2	3
434	x	x		2	1
502	x			1	2
516	x	x	x	3	1
602	x	x		2	0
639			x	1	0
667	x			1	1
Total	9	4	3		

Green Table No. (19).	Favorable reports only.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
4	x	x	x	3	10
65	x		x	2	2
85		x	x	2	2
102	x		x	2	0
103	x		x	2	6
105	x	x	x	3	1
107	x	x	x	3	7
169	x			1	1
399	x			1	0
435	x	x	x	3	4
462	x	x	x	3	2
467	x	x		2	0
477			x	1	0
512	x		x	2	3
517	x	x	x	3	5
521	x		x	2	0
599		x	x	2	0
600	x			1	0
692			x	1	3
Total	15	9	15		

Green Table No. (24).	Conflicting reports.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
8	x			1	5
9			x	1	1
13	x			1	6
15	x			1	0
55	x	x		2	2
84		x		1	2
86	x		x	2	8
88	x			1	0
95	x			1	2
106	x	x		2	5
138	x			1	0
160	x			1	0
163	x			1	0
197	x			1	4
427	x		x	2	2
428	x		x	2	3
448	x	x	x	3	4
451	x	x	x	3	5
457		x		1	0
480	x		x	2	1
504	x	x		2	5
563			x	1	0
584	x			1	1
601			x	1	1
Total	19	7	9		

Summary of recommendations from three sources—Continued.

Green Table No. (51).	Not reported on.			Number recommending.	Dealers offering.
	Ernst.	White Cross.	Beythien and Hempel.		
7	x			1	0
44	x			1	0
54			x	1	1
56	x		x	2	1
57	x		x	2	0
62	x			1	0
63	x		x	2	0
104	x			1	1
108	x			1	2
114	x			1	0
133	x			1	0
144	x		x	2	0
145	x			1	0
146	x			1	2
147	x			1	0
148	x			1	0
150	x			1	0
151	x			1	0
157	x			1	0
170	x	x		2	0
171	x			1	0
184	x			1	0
198	x			1	0
244	x			1	0
426	x			1	0
440	x	x		2	1
442	x	x		2	0
454	x		x	1	0
464	x	x		2	1
465	x	x		2	0
468	x	x		2	1
470	x	x		2	0
471	x	x		2	0
472	x	x		2	0
474	x	x		2	0
496	x			1	0
497	x			1	0
498	x			1	0
505	x			1	0
507	x	x		2	0
510	x			1	4
513			x	1	0
514	x		x	2	0
515	x		x	2	0
518			x	1	2
582			x	1	0
583	x			1	0
586	x			1	0
603			x	1	0
640			x	1	0
666	x			1	0
Total	45	11	13		

These three recommendations embrace 105 different Green Table numbers. The following table discloses the conformity of the composite of these three recommendations to the United States market in the summer of 1907:

Number of suggested samples in the three supplementary lists found on the market, 1907.

Data.	Total in class.	On United States market.
Unfavorable	11	7
Favorable	19	13
Conflicting	24	17
Not reported	51	10
Total	105	47

UNPUBLISHED RECOMMENDATIONS OF A MANUFACTURER.

In addition to these published recommended lists the following recommendation has been made by letter by a manufacturer who contributed specimens of food colors to the United States market in the summer of 1907:

That for the following permitted colors of Food Inspection Decision No. 76, there be substituted certain colors, the Green Table numbers alone being here given:

Permitted.	Substitute.
4	94
56	53
85	15
517	521

In regard to these proposed substitutes it is to be said that Nos. 15 and 521 were not on the United States market in the summer of 1907, according to the canvass made and described in Section I, Nos. 53 and 94 were on that market, No. 53 had not been examined physiologically, and No. 94 had been examined physiologically with only unfavorable results. The way was, therefore, not open to placing any of these colors on the permitted list under the procedure adopted. However, had No. 15 been on the market it would not have been placed on the permitted list, because it has been examined physiologically with contradictory results, while No. 521 might have been placed on the permitted list because it seems to have been examined physiologically and with only favorable results.

BÉHAL.

As the result of careful investigation Béhal (*Revue Générale des Matières Colorantes, 1910, p. 131*) suggests the use of 21 definite chemical individuals which are given in the following. With the tabulation on page 63 as a guide these 21 colors are classified as unfavorable, favorable, contradictory, and not reported on. The italicized figures are in the permitted list of Food Inspection Decision No. 76.

UNFAVORABLE (1).

425. Auramin O (3).

FAVORABLE (11).

4. Naphthol Yellow S (10).
65. Bordeaux B (2).
85. Orange I (2).
105. Fast Red (1).
107. Amaranth (7).
435. Light Green S F yellowish (4).
462. Acid Magenta (2).
467. Acid Violet 6B (none).
512. Eosin (2).
517. Erythrosin (5).
520. Rose Bengal (2).

CONTRADICTORY (6).

55. Ponceau 2 R (2).
84. Chrysoin (2).
106. New Coccin (5).
427. Malachite Green (2).
451. Methyl Violet (5).
480. Water Blue (1).

NOT REPORTED ON (3).

54. Scarlet R (1).
64. Crystal Ponceau (1).
440. Patent Blue (1).

The five in the "favorable" list which were on the United States market in the summer of 1907, but are not in the permitted list of Food Inspection Decision No. 76, namely:

65. Bordeaux B (2);
105. Fast Red (1);
462. Acid Magenta (2);
512. Eosin (2);
520. Rose Bengal (2),

are tinctorially provided for in that list; only one of the 21 colors recommended by Béhal was not on the market of the United States in the summer of 1907, namely, 467 Acid Violet 6B, and this is in the "favorable" list. The reasons for the non-inclusion of these colors are given on page 167.

CONCLUSIONS.

It is clear from an inspection of the preceding analysis of the six lists just given that the confusion referred to on page 165 as existing in the three recommendations made before the formulation of the permitted list of Food Inspection Decision 76 is not at all diminished by these six lists published after the announcement of that decision, as is shown in the following table:

Analysis of six lists published after the issuance of F. I. D. 76.

List of—	Total of dyes wanted.	Reports on physiological effect are—			Physiological effect not reported.	Number of dyes wanted and contained in F. I. D. 76.
		Unfavorable.	Favorable.	Contradictory.		
Ernst	88	9	15	19	45	4
Muttelet's class I	7	....	7	....	....	2
White Cross	31	4	9	7	11	5
Beythien and Hempel	40	3	15	9	13	6
Manufacturer	4	1	1	1	1	0
Béhal	21	1	11	6	3	5

The same wide differences of opinion as to the four points discussed on page 166 not only continue but are accentuated.

XIV. CHEMICAL EXAMINATION OF THE SEVEN PERMITTED COLORS, 1907.

NEED OF CHEMICAL CONTROL.

The most striking thing, from the chemist's point of view, in the literature relative to the physiological action of coal-tar colors, is the almost universal absence of the results of chemical examination or identification of the materials subjected to physiological test. There is hardly any description or statement of the strength or the concentration of the materials examined, or of the amount or nature of materials not coloring matter present in the substances subjected to these physiological tests. It is true that some publications give identification tests, but few assert the identity of the material subjected to certain physiological tests, with the description so given.

The need for some such chemical control as to identity and quality must be apparent to all having experience with the commercial varieties or grades of coal-tar colors. Many of these commercial brands of coal-tar colors contain added coloring matter other than the principal constituent for the purpose of correcting some defect unavoidably arising during the manufacture. Where those commercial brands are sold for textile and other manufacturing purposes there is no valid objection to such practice, because in such uses the tinctorial properties and effects of the commercial brands are the things the buyers desire and pay for, and the exact nature of the materials accomplishing the results is in reality of secondary importance; but when coal-tar colors are to be used in foods, and in addition to tinctorial effect the absence of any physiological action is necessary, it is at least of doubtful propriety to market such corrected or adjusted brands unless the correction or the adjustment be made with harmless colors.

There are a few instances in the relevant literature where it was attempted to explain the different results attained by observers by the difference in the composition of the materials subjected to physiological test, and there are other instances showing the difficulties encountered in obtaining specimens of coal-tar colors which would always give favorable physiological result.

The variation in composition of coal-tar colors may be exemplified by the following excerpts from the literature:

1. Weyl (*p. 91*), discussing the differences between his results and those of Cazeneuve and Lépine with Naphthol Yellow S, states as follows:

> It is to be noticed, however, that Cazeneuve and Lépine state that the Naphthol Yellow used (Jaune NS) was difficultly soluble, so that they probably experimented

> with another substance. My own investigations were made on dogs with a preparation for which I am indebted to the kindness of Dr. G. Schultz, of the Aniline Manufacturing Co. of Berlin. It was purified by precipitation and recrystallization.

2. Fraenkel (*p. 578*) expresses the opinion that the harmful results observed with Metanil Yellow (95 of the Green Tables) may be due to diphenylamin contained in the color, which diphenylamin may be a decomposition product of the Metanil Yellow, particularly since, as Weyl states (*p. 130*), it "smelled strongly of diphenylamin."

3. Pfeffer (*Unterschungen aus dem Botanischen Institut zu Tuebingen, Vol. II, p. 186*) says:

> In repeating my experiments I beg that it be considered that differences in observation may be caused by the quality of the coloring matters. For quite apart from the fact that the coloring matters are in part variable mixtures of various compounds, and not infrequently contain foreign admixtures, the quality of the goods brought into market may change from time to time. Thus formerly magenta was the acetate, whereas to-day it is the hydrochlorid. Also foreign admixtures may be poisonous or may exert no influence on the absorption of color.

4. Stilling (*Archiv. Exper. Path. Pharm., vol. 28, 1891, p. 352*) says:

> With respect to the blue pyoctanin, I have during the entire time been engaged in ascertaining the most effective. The things marketed by E. Merck as P. cœruleum have therefore now become uniform and homogeneous, which could not be the case at the beginning. The P. cœruleum now furnished by E. Merck is the hydrochlorid of pure hexamethylpararosanilin.

The variable degrees of purity attained by products of this kind on the market was apparently the first cause of the prohibition by the German Government of the coal-tar color known as Corallin for use in food products—not because Corallin itself was harmful, but because in the manufacture of this product it was so apt to retain impurities which in and of themselves produced bad effects, so that this particular color was specifically excluded by the German Government.

In a few cases it is stated that the substance subjected to physiological test had been purified, but these descriptions are hardly such as to enable others to arrive at substantially the same result with reasonable certainty.

5. Stilling in his monograph entitled Anilin Farbstoffe als Antiseptica (Strassburg, 1890, Pt. I, p. 16) says:

> The foregoing (relative to the action of Methyl Violet on the eyes of rabbits) holds only for pure substances. Many anilin colors of otherwise very antiseptic properties are contaminated with arsenic, particularly the otherwise useful Ethyl Violet. With such substance serious poisonings and death can be produced in experimental animals.

6. Stilling in Part II of the same monograph, pages 5 and 6, says:

> Such substances (coal-tar colors) must be chemically pure and can not, for example, be any mixture of various blue or violet dyes. There is therefore a great difference whether a substance be tested only bacteriologically or also in addition physiologically and therapeutically. Two preparations may be antiseptically wholly equal, but the one preparation may contain harmful admixtures which produce violent irritations.

7. Thus Weyl, speaking of his Metanitrazotin (*p. 120*), describes the purification method as follows:

> It is dissolved in warm alcoholic solution of sodium hydroxid filtered and precipitated with hydrochloric acid. The precipitate is freed from the adhering liquid by the aspirator and washed with hot water.

8. Weyl says of his Metanil Yellow (*p. 130*):

> For purification the color was dissolved in water filtered and separated by the addition of sodium acetate. The yellow mass was freed from the adherent liquid by the filter pump and dissolved in hot alcohol, in which it is difficultly soluble, and obtained from this in the form of yellow crystals. The material used for the experiment was almost pure, as the following analytical statement shows: 0.4895 gram of the color dried in 105° gave 0.084 sodium sulphate. Sodium required, 6.1; found, 5.6. (Note.—This amounts to 91.8 per cent of theory.)

9. Weyl (*Zts. Hygiene, 1889, Vol. II, p. 34,* On Safranin Poisoning) describes the difficulties he had in obtaining safranin on the market that was clean or pure; all preparations were free from arsenic, and contained small amounts of iron, chlorin, and traces of chromium. In one specimen the ash amounted to 4.8 per cent. The theoretical percentage of nitrogen in pure safranin is, according to Weyl, 15.3 per cent; in two commercial products he found 12.7 and 12.3 per cent, respectively (83 and 80.4 per cent of theory); he recrystallized the specimen containing 12.7 per cent twice from dilute hydrochloric acid; the first recrystallization produced a specimen containing 13.8 per cent (90.2 per cent of theory) of nitrogen; the second recrystallization produced a substance containing 14 per cent (91.5 per cent of theory) nitrogen.

10. Chlopin in his book (*p. 110*) says:

> Nevertheless, each dye was tested by me personally with the usual reagents and the dyeing of fabric in order to avoid the confounding of one dye with another. I convinced myself, from my experience, that not only druggists but the home offices of the makers occasionally send dyes which do not correspond to the requirements and resemble them only in name.
>
> (*Page 114.*) All the dyes, which according to my experiments proved to be poisonous, were carefully examined for contents of arsenic, chromium, and injurious metals, and were found to be free from these admixtures.

FIRST METHODS OF ANALYSIS USED.

In the summer of 1907 there were on the United States market 30 different specimens of the seven permitted colors of Food Inspection Decision No. 76. A chemical examination of those 30 specimens would disclose the qualitative conditions of the market so far as these seven permitted colors were concerned, and it was expected that certain limits for standards of cleanliness would be fixed by such an examination.

In examining these 30 specimens of the seven permitted colors chemically the following determinations were made:

1. Moisture.
2. Chlorin as chlorids.
3. Sulphated ash, together with its iron, aluminum, calcium, and copper content, and the determination of the sulphuric acid in the sulphated ash.
4. Total sulphur.
5. Gutzeit test (test 17 of the United States Pharmacopœia, eighth revision).
6. Heavy metals test (test 121 of the United States Pharmacopœia).
7. Total insolubles, together with the determination of the proportion that is volatile on ignition.
8. Ether extractive.

The methods of analysis actually used on these 30 dye specimens are here given solely for the purpose of comparing them with the methods developed therefrom and presented beginning with page 210. Experience has shown that the methods here given are defective in many particulars, and therefore they are not to be used for exact work.

MOISTURE.

Dry a sample of each color weighing 3 grams at 105° to 108° C. for two hours. The loss in weight is assumed to be moisture.

This method is not wholly accurate in the case of Naphthol Yellow, nor is it accurate in the case of Amaranth; but the scarcity of material made it seem unwise, at this stage, to undertake any extended investigation as to the amount or nature of the heating required surely to expel all moisture. The results, therefore, while not as accurate as might be desired, are, for the purposes of this exploratory investigation, sufficiently accurate for the object for which they were undertaken.

CHLORIN AS CHLORIDS.

Gently heat samples weighing 0.1 gram with 2 grams of sodium carbonate, and after destroying the greater part of the organic matter add 0.1 gram of powdered potassium nitrate and gently heat the whole until the organic matter is entirely destroyed. After cooling treat the whole with small amounts of cold water and remove from the crucible; effect the solution of the whole by gently heating. After cooling bring the bulk to about 150 cc, cool to room temperature, slightly acidify with nitric acid, precipitate the chlorin with silver nitrate, and weigh as silver chlorid.

SULPHATED ASH.

Moisten half-gram samples with concentrated sulphuric acid, gently evaporate to dryness, and treat the residue with 5 cc of concentrated sulphuric acid and again evaporate to dryness; repeat the operation until a white ash results, when the whole is ignited to constant weight. After weighing take up the sulphated ash in boiling water (and if necessary, any undissolved material can be taken up with hydrochloric acid by treatment on a boiling-water bath.) Mix the two solutions and bring to a total volume of 200 cc. In one half thereof determine the contained sulphur by a precipitation with barium chlorid, and weigh as barium sulphate; in the other half determine iron and aluminum by precipitation with ammonia, ignite and weigh as ferric and aluminum oxid. No separation of any contained aluminum was undertaken in the filtrate from the iron; the lime was precipitated as oxalate. In the only case that copper was encountered it was precipitated as sulphid before taking out the iron or the calcium.

TOTAL SULPHUR.

Mix 0.2 gram of the sample with 4 grams of sodium carbonate and 0.5 gram of potassium nitrate, and ignite to complete the destruction of the organic matter; take up in hot water; acidify with hydrochloric acid and precipitate the sulphur as barium sulphate with barium chlorid.

GUTZEIT TEST.

Mix 2 grams of the substance with 2 grams of a mixture of 1 part of potassium nitrate and 5 parts of sodium carbonate and ash in a porcelain crucible over a low flame; if not white when cool, mix the ash with 1 gram of potassium nitrate and again ash over a low flame. Generally the second ashing is sufficient. Dissolve the residue in 50 cc of hot water, boil, filter, neutralize with dilute sulphuric acid, and evaporate to substantial dryness on a boiling-water bath. Then add 1 cc of concentrated sulphuric acid and dry over a Bunsen flame; take up the residue with 5 cc of distilled water containing 0.5 cc of concentrated sulphuric acid and 10 cc of a saturated solution of sulphurous acid; evaporate the whole to a bulk of 5 cc on a water bath; add 20 cc of 8 per cent hydrochloric acid and subject this material to the action of 2 grams of metallic zinc, free from arsenic, which has been so activized by means of platinic chlorid that at the end of two hours more than 1 gram of zinc has dissolved and the evolution of gas has been constant and continuous. Conduct the reaction in a flask of 60 cc capacity with a neck 1 cm in diameter and 6 cm long. After the introduction of the solution and the zinc into the flask, stopper the neck of the bottle with gauze, the lower half of which is dry and the upper half moistened with the test solution of lead acetate of the United States Pharmacopœia. After carefully wiping the lip of the flask, cover it with Schleicher & Schüll quantitative filter paper which has been saturated three times with alcoholic mercuric chlorid solution, with complete drying between each saturation on one and the same spot of the filter paper.

In the case of Naphthol Yellow S it is necessary to heat gently with 10 grams of sodium carbonate until the organic matter is substantially all destroyed, then add 1.5 grams of potassium nitrate and heat to complete destruction of the organic matter. Dissolve the fused mass in hot water and a few drops of fifth-normal sulphuric acid; then make distinctly acid with the same and add an excess of 1 cc of concentrated sulphuric acid; evaporate first on the water bath and afterwards on asbestos until all odor of nitrous fumes and of hydrochloric acid has disappeared. Take up the residue in 5 cc of water and 15 cc of a saturated solution of sulphurous acid in water. Heat the whole on the water bath until no odor of sulphur dioxid remains.

The preparation of the sample by ignition with carbonate and nitrate should not be done in platinum, but should be done in a porcelain crucible, since it has happened that as much as 0.05 mg of arsenic mixed with the dye, which had been fused in platinum with carbonate and nitrate, could not be detected on the mercury-chlorid paper, whereas 0.01 mg when similarly treated in porcelain could always be detected, and 0.005 mg would usually be found when done in porcelain.

HEAVY METALS.

Mix as much of the substance as approximately contains 1 gram of color with 10 times its weight of carbonate of soda and ignite with the addition of 0.5 gram of potassium nitrate. Dissolve the whole in water, any undissolved material being taken up with hydrochloric acid, bring the two solutions together and slightly acidify with hydrochloric acid; dilute to 100 cc; place 10 cc in a test tube of 40 cc capacity and warm to 50° C. in a water bath; add 10 cc of a freshly prepared saturated solution of hydrogen sulphid in water, stopper the test tube well and allow the whole to stand in water having a temperature of 35° C. for a half hour. Dilute a 3.3 cc portion to 10 cc, treat as before with 10 cc of hydrogen sulphid in water for one-half hour at 35° C., and add to each ammonium hydrate.

TOTAL INSOLUBLES.

Dissolve 1 gram of the substance in 1 liter of water, filter through counterpoised quantitative filters, and wash with hot water until all traces of color are removed from the filter paper, then dry at 100° C. to constant weight and weigh; report the weight as total insolubles. Ignite the total insolubles in a platinum crucible and report the residue remaining as nonvolatile insolubles.

ETHER EXTRACTIVE.

Two methods were employed, the one consisting in direct extraction of the substance in a Soxhlet apparatus by means of redistilled ether dried over sodium. Schleicher & Schüll extraction cartridges were used after they had been thoroughly extracted by ether and shown by examination that they yielded no extractive to ether whatever. This is a necessary precaution, because the amount of ether extractive matter in these cartridges varies. The amount of ether extractive was determined by driving off the ether over a 32-candlepower incandescent lamp, finally drying and cooling in a desiccator.

This method, however, is not satisfactory, since the results it gives are undoubtedly low and it seems that the higher the material was dried the more erratic were the results. Therefore the following method was used:

Disolve 1 gram of the sample in 100 cc of water; add 0.5 gram of sodium acetate; extract three times with 50 cc of ether in a separatory funnel; mix the ether so recovered with 10 cc of water; separate the ether and dry with fused calcium chlorid; let stand from 12 to 24 hours; pour off from the calcium chlorid; distil off the ether as in the preceding method; acidify the color solution containing sodium acetate with 1 cc concentrated hydrochloric acid; extract three times with 50 cc of ether, and proceed as before.

RESULTS OF CHEMICAL EXAMINATION, 1907.

DETAILED CHEMICAL DATA ON EACH PERMITTED COLOR.

The results of this examination are given in the following tabulations under the respective Green Table numbers; the serial numbers refer to the numbers assigned to the specimens as they were received. The abbreviations "p." and "n. p." opposite "Gutzeit test," and "Heavy metals test" stand for "pass," and "not pass," respectively, according as the specimen did or did not comply with those requirements; the entries opposite "Ratio," indicate the degree of concordance of the sulphated ash as weighed, and the contained SO_3 calculated back to sodium sulphate and is a measure of the accuracy or dependability of the sulphated ash item and its determination.

As a guide in ascertaining the conformity of these colors to their supposed standards when judged by the analytical data obtained, the following table may of service:

Percentages of sulphur, sodium, and sulphated ash properly belonging to each of the seven permitted colors.

Green Table No.	Name of colors.	Sulphur.	Sodium.	Sulphated ash.
4	Naphthol Yellow	8.95	12.87	39.73
56	Ponceau 3 R	12.97	9.33	28.80
85	Orange I	9.16	6.58	20.31
107	Amaranth	15.92	11.44	35.32
435	Light Green	11.12	8.31	25.64
517	Erythrosin	(1)	5.24	16.17
692	Indigotin	13.75	9.89	30.52

[1] Iodin in No. 517—57.7 per cent.

Detailed analytical data obtained on several samples of each of the permitted colors (per cent).

GREEN TABLE NO. 4. NAPHTHOL YELLOW S.

Determinations.	Serial Nos.				
	23.	73.	108.	142.	187.
Moisture	0.70	0.28	0.28	0.60	0.33
NaCl	6.73	3.47	1.02	.61	2.86
Na as NaCl	2.65	1.37	.40	.24	1.13
Sulphated ash:					
Al and Fe	.07	.07	.07	.07	.28
Ca	.04	None.	None.	None.	None.
Na_2SO_4	38.00	48.8	47.1	51.4	49.2
SO_3 in Na_2SO_4=Na_2SO_4	38.18	49.57	47.32	51.76	49.44
Ratio	100.3	101.6	100.5	100.7	100.5
Na as Na_2So_4	12.32	15.82	15.46	16.66	15.95
Total sulphur	8.72	9.47	12.63	12.42	12.32
Gutzeit test	n.p.	n.p.	n.p.	n.p.	n.p.
Heavy metals test	n.p.	n.p.	n.p.	n.p.	n.p.
Total insolubles	.05	None.	None.	None.	.05
Nonvolatile	.03	None.	None.	None.	None.
Volatile	.02	None.	None.	None.	.05
Acetate ether extract	.34	.15	.14	.16	.10
Acid ether extract	.98	.90	.70	.75	.32
Total ether extract	1.32	1.05	.84	.91	.42
Ether extract solid	.062	.042	.04	.03	.022

Determinations.	Serial Nos.				
	201.	209.	228.	272.	280.
Moisture	0.50	0.60	0.32	2.12	9.07
NaCl	23.67	4.08	2.86	3.26	9.39
Na as NaCl	9.31	1.61	1.13	1.28	3.70
Sulphated ash:					
Al and Fe	.21	None.	.07	.07	.07
Ca	.03	None.	None.	None.	.07
Na_2SO_4	49.20	53.10	55.60	45.60	40.20
SO_3 in Na_2SO_4=Na_2SO_4	49.75	53.76	56.45	45.80	40.14
Ratio	101.1	101.3	101.5	100.4	99.84
Na as Na_2SO_4	15.95	17.22	18.03	14.79	13.03
Total sulphur	6.35	12.56	13.42	10.50	7.55
Gutzeit test	n.p.	n.p.	n.p.	p.	p.
Heavy metals test	n.p.	n.p.	n.p.	n.p.	n.p.
Total insolubles	.30	.80	.12	.90	1.30
Nonvolatile	.20	None.	.10	None.	.04
Volatile	.10	80	.02	.90	1.16
Acetate ether extract	.14	.25	.08	.15	.10
Acid ether extract	.33	1.08	.90	.72	.77
Total ether extract	.47	1.33	.98	.87	.87
Ether extract solid	.10	.004	.13	.04	.02

Detailed analytical data obtained on several samples of each of the permitted colors (per cent)—Continued.

GREEN TABLE NO. 56. PONCEAU 3R.

Determinations.	Serial No. 9.	Determinations.	Serial No. 9.
Moisture	5.76	Ratio	101.20
NaCl	19.89	Na as Na_2SO_4	12.36
Na as NaCl	7.84	Gutzeit test	p.
Sulphated ash:		Heavy metals test	n. p.
Al and Fe	.28	Acetate ether extract	.28
Ca	None.	Acid ether extract	.83
Na_2SO_4	38.10	Total ether extract	1.11
SO_3 in Na_2SO_4—Na_2SO_4	38.56	Ether extract solid	.328

GREEN TABLE No. 85. ORANGE I.

Determinations.	Serial Nos.		Determinations.	Serial Nos.	
	16.	224.		16.	224.
Moisture	11.06	5.39	Total sulphur	10.12	8.34
NaCl	8.78	3.51	Gutzeit test	p.	n.p.
Na as NaCl	3.18	1.38	Heavy metals test	p.	p.
Sulphate ash:			Total insolubles	1.50	.55
Al and Fe	.14	.07	Nonvolatile	.20	.20
Ca	None.	.14	Volatile	1.30	.35
Na_2SO_4	30.80	18.00	Acetate ether extract	.02	.62
SO_3 in Na_2SO_4—Na_2SO_4	31.18	18.09	Acid ether extract	.20	.20
Ratio	101.3	100.50	Total ether extract	.22	.82
Na as Na_2SO_4	9.99	5.84	Ether extract solid	.214	.254

GREEN TABLE No. 435. LIGHT GREEN SF YELLOWISH.

Determinations.	Serial Nos.			
	57.	92.	168.	233.
Moisture	8.32	5.08	5.04	4.15
NaCl	.041	.102	1.735	1.530
Na as NaCl	.016	.040	.683	.602
Sulphate ash:				
Al and Fe	.70	.28	.14	.14
Ca	3.29	.00	.085	.085
Na_2SO_4	9.00	43.40	48.00	53.00
SO_3 in Na_2SO_4—Na_2SO_4	7.78	43.96	46.68	53.62
Ratio	86.46	101.30	95.92	101.3
Na as Na_2SO_4	2.92	14.07	15.56	17.19
Total sulphur	11.48	15.44	15.82	16.75
Gutzeit test	Tr. (p.)	Tr. (p.)	Heavy.	Heavy.
Heavy metals test	p.	p.	p.	p.
Total insolubles	.20	.45	.75	.95
Nonvolatile	None.	.06	.60	.60
Volatile	.20	.39	.15	.35
Acetate ether extract	.02	.02	.06	None.
Acid ether extract	.05	.05	.05	.05
Total ether extract	.07	.07	.10	.05
Ether extract solid	.026	None.	.02	.02

GREEN TABLE No. 107. AMARANTH.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Moisture	4.24	6.46	6.23	9.81	4.20	8.24
NaCl	38.91	12.79	35.79	25.39	28.45	24.21
Na as NaCl	15.32	5.04	14.10	10.00	11.21	9.54
Sulphate ash:						
Al and Fe	.14	.07	.14	.42	.42	.21
Ca	.215	.857	.93	.428	.428	.57
Na_2SO_4	65.20	53.20	59.80	48.80	60.00	49.80
SO_3 in Na_2SO_4—Na_2SO_4	66.50	55.60	60.30	49.52	61.26	50.72

Detailed analytical data obtained on several samples of each of the permitted colors (per cent)—Continued.

GREEN TABLE No. 107. AMARANTH—Continued.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Ratio	102.00	100.80	100.80	101.5	102.1	101.9
Na as Na_2SO_4	21.13	17.22	19.39	15.82	19.45	16.14
Total sulphur	8.62	13.02	8.57	9.42	11.09	8.54
Copper	None.	None.	None.	.09	None.	None.
Gutzeit test	n. p.	n. p.	n. p.	n. p.	n. p.	n. p.
Heavy metals test	n. p.	n. p.	n. p.	n. p.	n. p.	n. p.
Total insolubles	.30	1.50	2.05	1.05	.90	2.40
Nonvolatile	.12	1.10	1.10	.35	.35	1.15
Volatile	.18	.40	.95	.70	.55	1.25
Acetate ether extract	.08	.15	.18	.20	.02	.30
Acid ether extract	.07	.15	.15	.03	.03	.10
Total ether contract	.15	.30	.33	.23	.05	.40
Ether extract solid	.024	.062	.052	.040	.044	.054

GREEN TABLE NO. 517. ERYTHROSIN.

Determinations.	Serial Nos.			
	184.	200.	216.	254.
Moisture			9.6	
NaCl (not determined)				
Sulphated ash:				
Al and Fe	0.14	0.14	.14	0.28
Ca	None.	None.	None.	None
Na_2SO_4	39.00	35.69	31.80	26.4
SO_3 in Na_2SO_4—Na_2SO_4	39.44	35.44	32.46	26.8
Na as Na_2SO_4	12.65	11.52	10.40	8.56
Gutzeit test	p.	n. p.	p.	p.
Heavy metals test	p.	p.	p.	p.
Total insolubles	.60	.20	.65	.90
Nonvolatile	.04	None.	.20	.20
Volatile	.56	.20	.45	.70
Acetate ether extract	.80	.35	.10	.10
Total ether extract				
Ether extract solid	.024	.07	.02	.02

GREEN TABLE NO. 692. INDIGO DISULPHO ACID.

Determinations.	Serial Nos.		
	90.	195.	249.
Moisture	7.25	5.32	7.31
NaCl	7.02	28.66	7.02
Na as NaCl	2.77	11.29	2.77
Sulphated ash:			
Al and Fe	.42	.28	.56
Ca	.57	.14	.71
Na_2SO_4	26.20	55.40	26.60
SO_3 in Na_2SO_4—Na_2SO_4	26.74	56.64	27.36
Ratio	102.10	102.20	102.8
Na as Na_2SO_4	8.50	17.97	8.63
Total sulphur	10.30	10.30	10.09
Gutzeit test	p.	p.	p.
Heavy metals test	p (?)	p (?)	p (?)
Total insolubles	1.55	.80	1.15
Nonvolatile	.55	None.	.50
Volatile	1.05	.80	.65
Acetate ether extract	.02	.02	.02
Acid ether extract	.05	.10	.03
Total ether extract	.07	.12	.05
Ether extract solid	.002	.014	.02

RECALCULATION OF ANALYTICAL DATA ON BASIS OF COLORING MATTER PRESENT.

The difficulties in the way of translating these analytical data into proximate constituents are so great, in so many of the cases, as to make any attempts to obtain practical results in that way absolutely useless; the amount of material on hand was unfortunately so small at the beginning (2 ounces or less, in most instances) that the utmost economy of material was necessary to get the data reported, on account of the large amount of material needed in the exploratory work done in trying out the methods for determining arsenic, heavy metals, and ether extractives.

In the arsenic test the coloring matter, as a whole, was considered, and no attempt was made to get at the actual amount of real coloring matter in the exact weights of the material as a whole, used for such examination, and consequently the results are not translatable into actual weights of real color used. It is obvious that if 2 grams, or other weight of the substance as a whole, failed to pass any test when the whole amount was considered as though it were all color, the material could not possibly have passed those tests if amounts thereof, corresponding to the prescribed weights of actual color used, were taken; also it is clear that if the sample, as a whole, passed a given test, at a given weight thereof, it would not necessarily have passed that test had an amount thereof, corresponding to that same weight of actual color, been used.

The amount of actual or real coloring matter in 27 of these 30 specimens is not greater than shown in the following:

Per cent of actual coloring matter in 27 samples.

Name of color.	Serial No.	Per cent.	Serial No.	Per cent.
Naphthol Yellow S	23	91.20	201	75.56
	73	95.20	209	93.19
	108	97.86	228	95.72
	142	97.88	272	92.81
	187	96.34	280	79.47
Ponceau 3R	9	73.24		
Orange I	16	78.44	224	89.76
Amaranth	82	56.40	162	63.52
	96	78.95	177	66.40
	130	55.60	219	64.25
Light Green SF Yellowish	57	91.37	168	92.37
	92	94.30	233	93.82
Erythrosin	216	89.65		
Indigo Disulpho Acid	90	84.11	249	84.47
	195	65.10		

In order to make these analytical results comparable among themselves and with other analytical results later to be given, the items common salt, volatile and nonvolatile insolubles, total insolubles, acetate ether extract, acid ether extract, and total ether extract of the preceding tabulations have been recalculated in parts per 100 of actual or real coloring matter as just enumerated:

Analytical data recalculated to parts per hundred of coloring matter present.

GREEN TABLE NO. 4. NAPHTHOL YELLOW S.

Determinations.	Serial Nos.									
	23.	73.	108.	142.	187.	201.	209.	228.	272.	280.
Common salt	7.38	3.64	1.04	0.62	2.97	31.53	4.38	2.99	3.51	11.82
Insolubles:										
Volatile	.02	.00	.00	.00	.05	.13	.86	.02	.97	1.46
Nonvolatile	.03	.00	.00	.00	.00	.27	.00	.10	.00	.05
Total	.05	.00	.00	.00	.05	.40	.86	.12	.97	1.51
Ether extract:										
Acetate	.37	.16	.14	.16	.10	.19	.27	.08	.16	.13
Acid	1.07	.95	.72	.77	.33	.44	1.16	.94	.78	.97
Total	1.44	1.11	.86	.93	.43	.63	1.43	1.02	.94	1.10

GREEN TABLE NO. 56. PONCEAU 3R.

Determinations.	Serial No. 9.	Determinations.	Serial No. 9.
Common salt	27.16	Ether extract:	
Insolubles:		Acetate	0.38
Volatile	....	Acid	1.13
Nonvolatile	....	Total	1.51

GREEN TABLE NO. 85. ORANGE I.

Determinations.	Serial Nos.		Determinations.	Serial Nos.	
	16.	224.		16.	224.
Common salt	11.19	3.91	Ether extract:		
Insoluble:			Acetate	0.02	0.69
Volatile	1.66	.39	Acid	.25	.23
Nonvolatile	.25	.23	Total	.27	.92
Total	1.91	.62			

GREEN TABLE NO. 107. AMARANTH.

Determinations.	Serial Nos.					
	82.	96.	130.	162.	177.	219.
Common salt	68.89	16.20	64.37	39.97	42.85	37.39
Insolubles:						
Volatile	.32	.51	1.71	1.10	.83	1.93
Nonvolatile	.21	1.39	1.98	.55	.54	1.78
Total	.53	1.90	3.69	1.65	1.37	3.71
Ether extract:						
Acetate	.14	.19	.32	.31	.03	.46
Acid	.12	.19	.27	.05	.05	.15
Total	.26	.38	.59	.36	.08	.61

Analytical data recalculated to parts per hundred of coloring matter present—Continued.

GREEN TABLE NO. 435. LIGHT GREEN SF YELLOWISH.

Determinations.	Serial Nos.			
	57.	92.	168.	232.
Common salt	0.04	0.10	1.88	1.64
Insoluble:				
Volatile	.22	.41	.16	.38
Nonvolatile	.00	.06	.65	.64
Total	.22	.47	.81	1.02
Ether extract:				
Acetate	.02	.02	.05	.05
Acid	.05	.05	.05	.05
Total	.07	.07	.10	.10

GREEN TABLE NO. 517. ERYTHROSIN.

Determinations.	Serial No. 216.	Determinations.	Serial No. 216.
Common salt	....	Ether extract:	
Insoluble:		Acetate	0.11
Volatile	0.50	Acid	....
Nonvolatile	.22	Total	.11
Total	.72		

GREEN TABLE NO. 692. INDIGO SULPHO ACID.

Determinations.	Serial Nos.			Determinations.	Serial Nos.		
	90.	195.	249.		90.	195.	249.
Common salt	8.35	44.02	8.31	Ether extract:			
Insoluble:				Acetate	0.02	0.03	0.02
Volatile	1.25	1.23	.77	Acid	.06	.15	.04
Nonvolatile	.59	.00	.59	Total	.08	.18	.06
Total	1.84	1.23	1.36				

MARKET QUALITY OF THE SEVEN PERMITTED COLORS.

That the quality of the lots of the seven permitted colors of Food Inspection Decision No. 76 offered on the markets of the world after the issuance of that decision was no better than that of the lots just reported, if as good, appears from the paper of E. G. Kohnstamm entitled, "Certified Food Colors: The Difficulties in the Way of their Manufacture," presented to the Seventh International Congress of Applied Chemistry held in London, May and June, 1909, and published in abstract form. This abstract reads as follows:

> The coal-tar colors permitted under the food and drugs act of the United States are seven in number, which must be in a high state of purity. The author states that none of the colors on the markets of the world, at the time of testing, would meet these requirements.

Of these seven colors, 189 samples, from every possible source, and representing all the leading manufacturers, are here reported on, and the results of their examination reasonably establish the necessity of food color certification. Of these 189 samples, the worst were offered for food coloring purposes and seemed to be so offered because unfit for any other purpose.

Naphthol Yellow S.—Sixty-four samples examined, ranging in shade from clear bright yellow to a dirty brownish or green color. All contained Martius Yellow, some more than 1 per cent thereof; some contained as high as 2 per cent and even 3 per cent unconverted initial material or decomposition products. Forty-one contained excessive arsenic and 29 excessive heavy metals.

Orange I.—Twenty-eight samples examined; all contained decomposition products varying from a slight amount to over 50 per cent; free α-naphthol was found in most samples; in 12 it was as high as 2 per cent; insoluble matters were as high as 1 per cent; lead to the extent of 0.5 per cent was found in one sample; shading by added colors and excessive amounts of arsenic; lead and iron were frequent.

Amaranth.—Thirty-eight samples examined; none were pure, and all contained arsenic in excessive amounts, and in one case as high as 0.1 per cent; all contained added color, principally an acid-violet. Iron as high as 0.1 per cent; insoluble matter as high as 1½ per cent; was most heavily loaded with salt of all seven colors.

Ponceau 3R.—Thirty-six samples examined; the purest of all colors tested; not toned; heavily loaded with salt; much insoluble matter was present; decomposition products were absent; iron, 0.01 per cent; 12 contained excessive amounts of arsenic and 14 contained excessive amounts of heavy metals.

Erythrosin.—Twelve samples examined; 10 were not erythrosin at all; of the other two, one was low in iodin and one contained arsenic.

Light Green S F Yellowish.—Thirteen samples examined; only one free from arsenic; nine contained lead or copper; one contained manganese; none were loaded.

Indigo Disulphonic Acid.—Eight samples examined; none were pure; the iron content was as high as 1.5 per cent; all were loaded with salt or Glauber's salt. One contained excessive amount of arsenic and two excessive amounts of heavy metals.

The difficulties consist in keeping the undesirable materials out of the dyes or in separating them from the crude dyes, or both.

In this connection the following statement made by Dr. E. Ludwig, of Vienna, at the International Congress of Medicine held in Budapest, August, 1909, may be of interest:

> The author, at an order of a court, at the beginning of the seventies in the last century, examined approximately 200 samples of food products confiscated as suspicious and taken from numerous sales places of a then suburb of Vienna; these samples included solid confectionery, fruit sirups, spirits, etc. He found that more than 90 per cent of these things were colored with magenta and contained arsenic. In some of the sales places the preparation used for coloring, the so-called "couleur," was found, which proved to be a solution of magenta and in which there were contained 8 per cent of arsenic in the form of arsenous acid and of arsenic acid. According to the statements of a qualified dyestuff maker, this "couleur" was a mother liquor from magenta manufacture, which was very difficultly saleable and which, however, a conscienceless agent had talked onto ignorant producers of and dealers in foods.

Schacherl (*p. 1046*) says: "It should be required of all permitted coloring matters that they shall not contain substances which are harmful to health, or even suspicious, either in chemical union or as contaminations." The following pages (Section XV) show how closely this requirement has been met as a result of quality control on the part of the Department of Agriculture.

XV. GUIDES IN DETERMINING DEGREE OF PURITY AND CLEANLINESS.

In view of the absence of any statements in the literature defining the purity of the colors physiologically examined with such accuracy that another could obtain with reasonable certainty the same degree of cleanliness, it became necessary to devise some guide, no matter how empirical, in the setting up of standards, tentative or otherwise.

As a first consideration it was held that, in view of the fact that all of the physiological work had been done with specimens of coal-tar colors of commercial purity, it would be reasonable to suppose that coal-tar colors produced in the purest form possible with present-day methods would certainly be as clean and as free from admixture as any of the commercial products subjected to physiological test. It was considered unreasonable to expect that increasing the degree of purity of these substances could in any way increase any harmful property possessed by them. Certainly in the case of Naphthol Yellows, where Martius Yellow is a usual contaminant, it can hardly be maintained that decreasing the amount of Martius Yellow would increase any harmful property which might reside in Naphthol Yellow S proper; in the case of Ponceau 3 R it could hardly be argued that any undisazotized base or decomposition products of diazo compounds tended to correct or counteract any harmful property that might reside in Ponceau 3 R proper; nor could it be maintained that Orange I free from uncombined alpha-naphthol was more harmful than Orange I, contaminated with alpha-naphthol, and so on through the list of the seven permitted colors. This point would not be raised had it not been pressed repeatedly by different persons as a serious objection to quality control and purity standards of the seven permitted colors of Food Inspection Decision No. 76.

With this rule in mind, and referring to the tabulated results of the analyses of the 30 specimens of the seven permitted colors just given, the items in the analytical statements will each be separately discussed.

Common salt and ether extractive.—Common salt is a legitimate component of commercial brands of coal-tar colors in so far as these coal-tar colors are obtained by the so-called "salting out" process. The coal-tar colors are recovered from solution by the addition of common salt, which has the peculiar property of separating the coal-tar color from the water solution as an undissolved solid. The coal-tar color so obtained will contain more or less salt, which, from a commercial manufacturing point of view, is therefore an unavoidable constituent. The amount of salt so accompanying the coal-tar color depends upon the amount added to the color solution, and this is greatest when complete exhaustion of the coal-tar color solution is attempted. It is a matter of common experience that, as a rule, the coal-tar color first separating in the salting-out process is cleaner and less contaminated than the portions last separating. In an endeavor to recover as far as possible all the dissolved coal-tar coloring matter the manufacturer adds a large excess of salt, and this carries with it a large amount of organic matter not coloring matter, as can be seen from the preceding analyses, where a high salt content is almost always accompanied by a high ether extract content. The ether extract content is a measure of the dirt or organic impurities of the coloring matter.

The objection to an excessive amount of salt in coal-tar coloring matters, when they are to be used for food-coloring purposes, does not reside in the salt per se, but is due to the fact that a high salt content seems generally to be accompanied by an unnecessarily large amount of organic material not coloring matter, and nothing good is known of such material, which is more than likely to be harmful.

The acetate ether extract is supposed to represent that part of the organic material not coloring matter which has no, or only slightly, basic properties; the acid ether extract is supposed to represent that part of the organic material not coloring matter which has acid properties. In the case of Naphthol Yellow S the acid ether extract will contain all of the Martius Yellow, and very likely will consist substantially of it. In the case of Erythrosin, of course, no acid ether extraction was attempted because the color acid is itself ether-soluble.

The following tabulation shows the percentages of salt based on the amount of coloring matter present contained in 26 of the specimens before reported on, arranged under each of the seven permitted colors:

Naphthol Yellow S: 0.62; 1.04; 2.97; 2.99; 3.51; 3.64; 4.38; 7.38; 11.82; 31.53. (Average, 6.99.)
Ponceau 3 R: 27.16.
Orange I: 3.91; 11.19. (Average, 7.55.)
Amaranth: 16.20; 37.39; 39.97; 42.85; 64.37; 68.89. (Average, 44.95.)
Light Green SF Yellowish: 0.04; 0.10; 1.64; 1.88. (Average, 0.92.)
Indigo Disulpho Acid· 8.31; 8.35; 44.02. (Average, 20.23.)

The following figures give the same data for the acetate, acid, and total, ether extract:

Ether extractives.

ACETATE ETHER EXTRACT.

Naphthol Yellow S: 0.08; 0.10; 0.13; 0.14; 0.16; 0.16; 0.16; 0.19; 0.27; 0.37. (Average, 0.18.)
Ponceau 3 R: 0.38.
Orange I: 0.02; 0.69. (Average, 0.36.)
Amaranth: 0.03; 0.14; 0.19; 0.31; 0.32; 0.46. (Average, 0.24.)

Light Green SF Yellowish: 0.02; 0.02; 0.05; 0.05. (Average, 0.035.)
Erythrosine: 0.11.
Indigo Disulpho Acid: 0.02; 0.02; 0.03. (Average, 0.03.)

ACID ETHER EXTRACT.

Naphthol Yellow S: 0.33; 0.44; 0.72; 0.77; 0.78; 0.94; 0.95; 0.97; 1.07; 1.16. (Average, 0.81.)
Ponceau 3 R: 1.13.
Orange I: 0.23; 0.25. (Average, 0.24.)
Amaranth: 0.05; 0.05; 0.12; 0.15; 0.19; 0.27. (Average, 0.14.)
Light Green SF Yellowish: 0.05; 0.05; 0.05; 0.05. (Average, 0.05.)
Indigo Disulpho Acid: 0.04; 0.06; 0.15. (Average, 0.08.)

TOTAL ETHER EXTRACT.

Naphthol Yellow S: 0.43; 0.63; 0.86; 0.93; 0.94; 1.02; 1.10; 1.11; 1.43; 144. (Average, 0.99.)
Ponceau 3R: 1.51.
Orange I: 0.27; 0.92. (Average, 0.59.)
Amaranth: 0.08; 0.26; 0.36; 0.38; 0.59; 0.61. (Average, 0.38.)
Light Green SF Yellowish: 0.07; 0.07; 0.10; 0.10. (Average, 0.09.)
Erythrosin: 0.11.
Indigo Disulpho Acid: 0.06; 0.08; 0.18. (Average, 0.11.)

Insoluble matter.—The amount of insoluble matter (total, volatile, and nonvolatile) is a measure of the cleanliness of the materials used, as well as of the cleanliness of treatment during the manufacture of the coloring matter. The following tabulation shows the variations in these three figures for each of the 26 specimens of the 7 permitted colors, examined, as in the preceding cases:

Insoluble matter.

VOLATILE INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.02; 0.02; 0.05; 0.13; 0.86; 0.97; 1.46. (Average, 0.35.)
Orange I: 0.39; 1.66. (Average, 1.03.)
Amaranth: 0.32; 0.51; 0.83; 1.10; 1.71; 1.93. (Average, 1.07.)
Light Green SF Yellowish: 0.16; 0.38; 0.22; 0.41. (Average, 0.29.)
Erythrosin: 0.50.
Indigo Disulpho Acid: 0.77; 1.23; 1.25. (Average, 1.08.)

NONVOLATILE INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.00; 0.00; 0.00; 0.03; 0.05; 0.10; 0.27. (Average, 0.05.)
Orange I: 0.23; 0.25. (Average, 0.24.)
Amaranth: 0.21; 0.54; 0.55; 1.39; 1.78; 1.98; (Average, 1.08.)
Light Green SF Yellowish: 0.00; 0.06; 0.64; 0.65. (Average, 0.34.)
Erythrosin: 0.22.
Indigo Disulpho Acid: 0.00; 0.59; 0.59. (Average, 0.36.)

TOTAL INSOLUBLE.

Naphthol Yellow S: 0.00; 0.00; 0.00; 0.05; 0.05; 0.12; 0.40; 0.86; 0.97; 1.51. (Average, 0.40.)
Orange I: 0.62; 1.91. (Average, 1.27.)

Amaranth: 0.53; 1.37; 1.65; 1.90; 3.69; 3.71. (Average, 2.14.)
Light Green SF Yellowish: 0.22; 0.47; 0.81; 1.02. (Average, 0.63.)
Erythrosin: 0.72.
Indigo Disulpho Acid: 1.23; 1.36; 1.84. (Average, 1.48.)

Work subsequent to these analyses showed that the insoluble matter, particularly the nonvolatile insoluble matter, was greater in certain batches than in others, and that these amounts were in excess of the maximum amounts reported therefor. Examination showed that these increased amounts of insoluble matter were probably due to variations in the water delivered to the factories by their respective municipal water supplies, since it was noted that in two or more cases the batches containing an exceptionally large amount of nonvolatile insoluble matter were manufactured at a time when the city water supply was abnormally hard; this item, therefore, has been made in some instances a little more elastic than the preceding analyses would seem to justify.

Arsenic.—Examination of the preceding tabulations shows that 14 out of 30 specimens tested passed the Gutzeit test for arsenic, and 16 failed to pass.

Heavy metals.—Inspection of the preceding tables shows that 13 out of 30 specimens passed this test, and that 17 failed to pass.

Combined arsenic and heavy metals test.—Inspection of the preceding tables shows that 11 out of 30 passed both tests jointly; that 3 passed the arsenic test and failed to pass the heavy metals test; and that 2 passed the heavy metals test and failed to pass the arsenic test.

Moisture.—The amount of moisture, as inspection of the preceding analyses shows, is variable, and is a factor not under easy control. As long as the coloring matters submitted for foundation certification were in powder form and the analyses disclosed the actual percentage of coloring matter it was considered that any control of this item would involve an amount of labor and provide opportunities for friction wholly out of proportion to any benefit that at present could be realized therefrom. Therefore such control was not instituted, although it by no means follows that the time may not come when control of this item will be necessary.

Sulphur and sulphated ash.—These two determinations in the case of all the permitted colors, except Erythrosin, give a rough measure of the extent of the sulphonation and of the saturation of the sulphonic acids with sodium. It is not the function of these two items to exclude the isomeric modifications, nor was any test applied to such of these 30 specimens as were examined in this respect to determine the presence or absence of such products. The attempts made in this direction are of a later date than the analyses already reported, and are indicated on page 210 of this report.

With these data available, and with the general rule stated at the opening of this section in mind, it was regarded as the proper course, when determining upon standards, to require that each color should pass the combined heavy metals and arsenic test of the United States Pharmacopœia, and in other respects should be as clean and as high-grade as the best of each class given in the preceding analyses. Just how closely it was possible to adhere to this rule will be shown later.

With respect to the arsenic requirement, there has been a great deal of discussion brought on by those interested in the manufacture of these colors. It was protested that colors could not be made uniformly and continuously under manufacturing conditions, which would contain an amount of arsenic so small that a quantity of the product containing 2 grams of the coloring matter in question would not respond to the Gutzeit test in the United States Pharmacopœia. The results above given with respect to conformity or nonconformity to the Gutzeit test, were, with a great deal of justification, not regarded as conclusive, because, as has been previously stated, there was no certainty that the amount of coloring matter actually taken did represent 2 grams. In view of the fact that the United States Pharmacopœia prescribes for the only coal-tar color in it, namely, Methylene Blue, that it shall be so free from arsenic that 2 grams fail to respond to the Gutzeit test, it was considered perfectly justifiable to adhere to that requirement until it could be conclusively shown that it was unreasonable and incapable of attainment. Results described in the pages that follow show that it has been possible to make all the 7 permitted colors of Food Inspection Decision No. 76 so free from arsenic that they comply with the Pharmacopœial test.

With respect to the heavy metals, no deviation was necessary for the nonferrous metals from the test of the Pharmacopœia; for iron, however, it was found necessary to increase the limit to substantially 0.005 part per 100 of actual coloring matter. The reason for this is that at one time or another all of the seven permitted coloring matters in the course of their manufacture come in contact with, or are contained in, vessels of iron, and it seems to be almost impossible to keep iron out to an extent which would bring the color within the pharmacopœial test. It has been shown that there were 13 colors on the market in 1907 which contained so little iron that they failed to respond to the heavy metals test of the Pharmacopœia in that respect; but here again the same criticism holds good as in the arsenic test, that there is no certainty that the amounts taken for the heavy metals tests were equivalent in each case to 1 gram of actual coloring matter, and subsequent experience seems to confirm the correctness of that criticism; therefore the rule for cleanliness of product, as just given, has been modified in that respect and to that extent.

That it was only fair and reasonable to expect considerable improvement in the cleanliness and purity of food colors was made evident by an examination of two substances sold in the United States in large quantities for the purpose of making a very cheap red coal-tar coloring matter, which is used in many of the cheapest coloring operations, for paints, inks, etc. These substances are paranitranilin and betanaphthol. They were found in the United States market in such a condition of cleanliness and purity that had they been suitable for use in foods no objection could be raised against them on these scores. They complied with the requirements of the United States Pharmacopœia with respect to freedom from arsenic and all heavy metals, inclusive of iron. The significance of this lies in the fact that all the raw materials entering into the manufacture of paranitranilin and betanaphthol also enter into the manufacture of the seven permitted colors of Food Inspection Decision No. 76, and that the only source of arsenic in food colors, and probably the only way in which iron could be introduced into them, is by way of the materials entering into the manufacture of paranitranilin and betanaphthol, and since it has been shown to be commercially possible to keep those bodies out of paranitranilin and betanaphthol, and since there is no occasion whatever for arsenic or iron or other heavy metal being present in any of the materials used in the manufacture of the seven permitted colors of Food Inspection Decision No. 76, over and above the materials used in the manufacture of paranitranilin and betanaphthol, there was every reason for believing that the seven permitted colors of Food Inspection Decision No. 76 could ultimately be manufactured and marketed in the same degree of cleanliness and purity that paranitranilin and betanaphthol are marketed; in other words, that food colors could be made as clean and as pure as paint colors, a condition not existing in the food-color market of the United States in the summer of 1907.

The results of the control exercised by the Department of Agriculture over the quality of food colors, as compiled in the following section, fully justify such expectations, as well as the aim to make coal-tar colors when used for food purposes of the same high degree of cleanliness and purity as when they are to be used as drugs or as they actually are when used for the manufacture of paints and printer's inks; that is to say, that the coal-tar color used in a colored food should be as clean as the coal-tar color used in making the ink on the label of such colored food, the very reverse of the situation existing prior to the quality control now established.

XVI. ANALYSES OF CERTIFIED LOTS OF PERMITTED COLORS, 1909-10.

TABULATION OF RESULTS.

The analytical results obtained on 74 batches of certified colors, totaling upward of 32,000 pounds, are given in the following tables. The analytical results are the work of the New York Food and Drug Inspection Laboratory in the course of checking up the analytical results certified to in foundation and in supplementary certificates. The period covered by these examinations is approximately from July, 1909, to January 1, 1910.

Naphthol Yellow S.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.]

Number of batches.	Insolubles.		Ether extractives.				Common salt.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1	0.170	0.100	0.036	0.016	0.067	0.088	4.06	9.23	13.27	0.13
2	.130	.078	.026	.007	.065	.073	2.26	8.99	13.21	.13
3	.097	.050	.024	.006	.042	.072	1.68	8.98	12.92	.06
4	.090	.046	.023	.006	.038	.064	1.41	8.96	12.90	.04
5	.082	.040	.019	.005	.036	.061	1.30	8.89	12.89	....
6	.079	.036	.018	.005	.034	.057	.63	8.86	12.89	....
7	.070	.032	.016	.004	.031	.056	.63	8.85	12.87	....
8	.060	.030	.012	.004	.026	.054	.48	8.83	12.87	....
9	.055	.010	.011	.000	.025	.048	.47	8.71	12.81	....
10	.050	.010	.010	.000	.025	.045	.25	8.70	12.75	....
11	.050	....	.007	.000	.020	.045	.24	8.62	....	....
12	.030	....	.006	.000	.018	.045	.19	....	....	....
13	.030	....	....	....	....	.044	.19	....	....	....
Average	.076	.043	.017	.004	.035	.051	1.06	8.87	12.93	.09
Above	6	4	6	6	5	8	5	5	2	2
Below	7	6	6	6	7	5	8	6	8	2

Ponceau 3R.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.	Boiling point of cumidin.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.						
												°C.
1	0.55	0.29	0.166	0.034	0.019	0.216	5.72	0.06	13.03	9.51	0.18	222-235
2	.42	.20	.163	.033	.018	.213	5.67	.06	12.99	9.49	.15	223-235
3	.41	.17	.161	.032	.018	.201	5.57	.00	12.95	9.31	.14	222-235
4	.27	.12	.072	.009	.018	.098	5.00	.00	12.88	9.31	.14	220-230
5	.17	.11	.053	.006	.017	.073	4.12	.00	12.80	9.23	.12	220-230
6	.17	.11	.052	.002	.017	.072	3.73	.00	12.80	9.16	.12	220-230
7	.16	.09	.038	.002	.016	.058	3.71	.00	12.77	9.12	....	220-229
8	.14	.07	.038	.000	.014	.057	3.03	.00	12.64	9.07	....	225-228
9	.10	....	....	....	....	....	2.53	.00	....	8.74	....	216-226
Average	.266	.15	.093	.015	.017	.124	4.34	.01	12.83	9.21	.14	221-231
Above	4	3	3	3	4	3	4	....	4	5	2	3
Below	5	5	5	5	4	5	5	....	4	4	4	6

Orange I.

[**Figures calculated to 100 parts pure color, and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.**]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1	0.39	0.18	0.224	0.093	0.08	0.360	3.13	0.22	9.26	6.82	0.29
2	.38	.08	.220	.060	.077	.309	3.13	.10	9.23	6.76	.24
3	.28	.08	.210	.043	.067	.306	3.04	....	9.12	6.63	.24
4	.23	.05	.207	.035	.055	.300	2.36	....	9.10	6.62	.22
5	.23	.04	.181	.035	.048	.269	1.98	....	9.06	6.54	.05
6	.16	.04	.177	.020	.039	.267	1.91	....	9.06	6.48	.04
7	.15	.03	.174	.018	.036	.246	1.90	....	9.05	6.32	....
8	.11	.03	.124	.015	.029	.224	1.41	....	9.02	6.21	....
9	.10	.02	.107	.011	.016	.175	1.05	....	8.96	....	....
10	.05	....	.097	.011	....	.124	1.01	....	8.15	....	....
Average	.21	.06	.172	.034	.050	.258	2.09	.03	9.00	6.55	.18
Above	5	3	7	5	4	6	4	1	8	4	4
Below	5	6	3	5	5	4	6	1	2	4	2

Amaranth.

[**Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.**]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1	0.43	0.33	0.090	0.030	0.059	0.158	4.18	16.04	14.22	0.20
2	.39	.30	.088	.017	.017	.101	4.16	15.80	14.19	.19
3	.29	.19	.069	.009	.015	.100	3.83	15.63	12.10	.17
4	.23	.15	.047	.007	.014	.060	3.73	15.61	11.47	.14
5	.21	.10	.042	.007	.012	.056	3.67	15.59	11.43	.14
6	.13	.10	.035	.005	.012	.053	3.08	15.58	11.43	.13
7	.12	.06	.034	.005	.012	.052	3.02	15.57	11.42	.13
8	.12	.05	.034	.005	.010	.051	2.94	15.57	11.38	.12
9	.12	.05	.034	.005	.009	.048	2.70	15.56	11.33	.12
10	.11	.04	.031	.004	.008	.042	2.62	15.54	11.32	.12
11	.11	.04	.031	.004	.008	.042	2.21	15.52	11.31	.11
12	.11	.040	.031	.004	.007	.040	2.12	15.52	11.29	.09
13	.11	.036	.027	.004	.006	.039	2.12	15.52	11.27	.09
14	.092	.03	.026	.004	.006	.038	2.04	15.47	11.27	.09
15	.09	.03	.026	.003	.005	.038	1.94	15.43	11.23	.09
16	.09	.025	.025	.002	.005	.038	1.94	15.41	11.21	.08
17	.089	.02	.021	.001	.004	.035	1.91	15.41	11.18	.07
18	.08	.02	.019	.000	.003	.035	1.91	15.41	11.15	.00
19	.070	.02	.018	.000	.003	.029	1.23	15.40	11.14	....
20	.07	.02	.012	.000	....	....	1.22	15.12	11.10	....
21	.04	....	....	....	....	....	....	15.12	11.01	....
Average	.148	.083	.037	.006	.011	.056	2.63	15.51	11.59	.012
Above	5	6	5	5	7	4	9	13	3	7
Below	16	14	15	15	12	15	11	8	18	11

Light Green SF Yellowish.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1	0.07	0.034	0.058	0.011	0.011	0.076	4.15	0.00	12.69	8.03	4.52
2	.045	.02	.041	.007	.009	.054	.64	.00	12.27	7.99	.10
3	.03	.01	.034	.006	.008	.053	.515	.00	12.07	7.99	.09
4	.02	.01	.033	.000	.007	.042	.43	.00	11.88	7.94	.06
5	.02	.01	....	....	....	....	.37	.00	11.88	7.73	.06
6	.01	.01	....	....	....	....	....	.00	....	1.56	....
Average	.033	.017	.041	.006	.008	.056	1.221	.00	12.16	6.87	.9
Above	2	2	1	2	2	1	1	0	2	5	1
Below	4	3	3	2	2	3	4	0	3	1	4

Erythrosin.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures in the same horizontal line do not refer to the same sample; the dash line shows the location o the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium sulphate.	Sodium.	Iodin.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.				
1	0.13	0.09	0.039	0.017	....	0.051	1.13	0.00	5.42	57.45
2	.09	.04	.039	.009	....	.048	1.00	.00	5.35	56.46
3	.059	.024	.037	.008	....	.047	.48	.00	5.33	56.40
4	.05	.01	.034	.002	....	.037	.23	.00	5.29	56.07
5	.04	.01	.033	.002	....	.035	.09	.00	5.15	55.96
6	.04	.01	.033	.000	....	.035	.09	.00	5.11	55.97
7	.04	.01	.027	....	....	.027	.09	.00	3.85	55.94
8	.03	....	....	....	....	....	.09	.00	....	55.90
9	....	....	....	....	....	....	.036	.00	....	55.88
Average	.059	.028	.035	.006	....	.040	.360	.00	5.07	56.23
Above	2	2	3	3	....	3	3	....	6	3
Below	6	5	4	3	....	4	6	....	1	6

Indigo disulpho acid.

[Figures calculated to 100 parts pure color and arranged in the order of their size. Figures on the same horizontal lines do not refer to the same samples; the dash line shows the location of the average of each column.]

Number of batches.	Insoluble.		Ether extractives.				Common salt.	Sodium-sulphate.	Sulphur.	Sodium.	Calcium.
	Total.	Inorganic.	Neutral.	Alkali.	Acid.	Total.					
1	0.50	0.28	0.140	0.087	0.099	0.320	6.77	16.12	13.45	10.13	0.45
2	.46	.26	.134	.061	.038	.217	3.60	10.22	13.43	9.90	.13
3	.45	.25	.102	.029	.026	.143	3.21	4.79	13.42	9.64	.15
4	.43	.24	.088	.015	.024	.141	2.89	.00	13.39	9.60	.05
5	.42	.22	.075	.013	.016	.126	1.85	.00	13.22	9.13	....
6	.32	.16	....	....	....	....	.40	.00	13.15	8.54	....
Average	.43	.235	.108	.041	.041	.189	3.12	5.19	13.34	9.49	.19
Above	3	4	2	2	1	2	3	2	4	4	1
Below	3	2	3	3	4	3	3	4	2	2	3

COMPARISON WITH ANALYSES MADE IN 1907.

A comparison, as far as possible, of these figures with the corresponding data previously given for samples on the market in the summer of 1907 is made in the following table. The figures in this new table show the value of the fraction obtained by dividing the old average figure by its corresponding new average figure—that is, they show how many times greater the old average figures are than the corresponding new average figures. Italics indicate those items in which there has been a retrogression in the new figures as compared with the old; all the other figures represent an advance. It will be noted that comparisons are not made for Ponceau 3R and Erythrosin. This comparison was omitted because there was but one sample of Ponceau 3R examined and a partial analysis of one sample of Erythrosin in the old work.

Ratio of average figures of 1907 to those for the certified colors, 1909.

Color.	Salt.	Insoluble.			Ether extracts.		
		Total.	Volatile.	Nonvolatile.	Nonacid.	Acid.	Total.
Naphthol Yellow S	4.88	4.44	9.02	*0.67*	7.50	21.89	15.97
Orange	3.54	9.07	20.60	3.00	1.57	4.53	2.26
Amaranth	15.07	11.26	21.40	7.71	4.07	10.77	5.00
Green	*.66*	31.00	29.00	17.00	*.65*	6.25	1.67
Blue	8.05	3.29	5.4	1.56	*.15*	3.48	*.68*

These retrogressions are:

1. Salt in the Green.
2. Nonvolatile insolubles in the Yellow.
3. Nonacid ether extract in the Green.
4. Nonacid ether extract in the Blue.
5. Total ether extract in the Blue.

With respect to the first of these retrogressions there is this to be said: One lot of Green had apparently been purified by precipitation with salt, since it was in every other respect of a high quality—that is, it was free from arsenic and heavy metals within the pharmacopœial test, and its ether extractives were very satisfactory. The other lots of Green examined had apparently not been made in this manner.

With respect to the second retrogression, the probable explanation is that some of the lots were made during a period when the municipal water supply was excessively hard, as before explained. The remaining three retrogressions are probably due to the fact that the old methods of analysis used were not so accurate as the methods later employed and hereinafter described (pp. 223, 225).

It will be noted that in three of the six batches of Indigo disulpho acid reported there is no sodium sulphate; whereas in the other three batches the amount of this substance is as high as 16.12. The reason for this is that in the early stages of the work the results obtained by the manufacturers pointed in the direction of the impossibility of getting rid of all the sodium sulphate; but later and more extended work showed this conclusion to be an error. There is therefore no good reason for permitting sodium sulphate as a contaminant in this or any other of the seven products.

CONFORMITY OF ANALYTICAL DATA WITH THEORETICAL COMPOSITION.

The seven tables following, likewise based upon the 74 Government analyses, show the conformity or nonconformity, as the case may be, of these samples with their theoretical composition. The second and third columns show the percentage based on theory of the contained sulphur and sodium in the case of all colors except Erythrosin, where the figures for iodin are substituted for those of sulphur. The fourth column shows the actual ratio of sodium to sulphur or iodin, as the case may be, and the fifth column gives the percentage of the figure of column 4 based on the theoretical value which is given in parentheses at the head of columns 4 and 5. The summary gives the maximum, minimum, and average of each column. Taken as a whole the figures are fairly satisfactory and show a reasonable conformity of the actual product to theoretical requirements, although there seems to be considerable room for improvement, which no doubt can be achieved in time.

Percentages based on theoretical composition.

NAPHTHOL YELLOW S.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(1.4379)$.		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(1.4379)$.	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1	103.120	103.10	1.4377	99.983	10	98.880	101.06	1.4576	101.36
2	96.312	99.068	1.4791	102.860	11	98.658	101.22	1.4631	101.74
3	97.205	100.000	1.4792	102.870	12	100.330	100.15	1.4353	99.826
4	97.542	99.534	1.4707	102.270	13	100.100	100.00	1.4363	99.890
5									
6	....	....	....	....	Average	99.101	10[illegible].212	1.4578	101.378
7	98.992	....	....	....	Max	103.120	103.500	1.4792	102.87
8	99.331	100.15	1.4498	100.80	Min	96.312	99.068	1.4353	99.826
9	100.440	103.50	1.4692	102.18					

PONCEAU 3R.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}=(0.71905)$.	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1	97.454	93.617	0.69138	96.173	8	98.693	101.920	0.74296	103.33
2	....	98.927	....	....	9	99.308	101.700	.73679	102.48
3	100.460	97.749	.69992	97.351					
4	100.140	98.174	.70518	98.080	Average	99.256	98.738	.71648	99.144
5	98.458	97.095	.70936	98.662	Max	100.46	101.920	.74296	103.33
6	99.841	99.783	.71892	99.918	Min	97.454	93.617	.69138	96.173
7	98.693	99.783	.72730	101.16					

Percentages based on theoretical composition—Continued.

ORANGE I.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}$=(0.71905).		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}$=(0.71905).	
			Value.	Per cent.				Value.	Per cent.
1........	88.972	100.44	0.81226	112.97	8........	98.471	85.901	0.70066	97.454
2........	100.76	103.72	.73889	102.77	9........	99.563	99.241	.71710	99.740
3........	101.09	102.53	.73002	101.53	10........	99.342	98.330	.71208	99.042
4........	99.135	....	....	....					
5........	99.135	....	....	....	Average.	98.308	99.376	.72957	101.475
6........	97.816	94.234	.69309	96.391	Max.....	101.09	103.72	.81226	112.97
7........	98.799	100.610	.73243	101.90	Min.....	88.972	95.901	.69309	97.454

AMARANTH.

1........	96.795	105.76	0.78519	109.21	14........	97.800	98.863	0.72640	101.03
2........	98.176	99.037	.72488	100.82	15........	97.499	99.911	.73646	102.43
3........	94.975	124.29	.94048	130.80	16........	97.614	98.950	.72843	101.32
4........	94.975	124.04	.93849	130.53	17........	97.499	99.476	.73325	101.98
5........	100.750	96.240	.68637	95.470	18........	97.863	98.689	.72465	100.78
6........	99.246	99.911	.72341	100.62	19........	98.052	98.393	.72106	100.05
7........	97.174	100.260	.72454	100.78	20........	97.800	98.514	.72383	100.68
8........	96.929	99.827	.74011	102.94	21........	97.735	97.725	.71852	99.914
9........	97.926	97.465	.71520	99.476					
10........	96.975	97.990	.72744	101.18	Average.	97.467	101.347	.74707	103.90
11........	96.733	97.389	.72338	100.62	Max.....	100.750	124.29	.94048	130.80
12........	97.487	98.514	.72615	100.99	Min.....	94.975	96.240	.68637	95.470
13........	96.798	97.028	.72030	100.18					

LIGHT GREEN SF YELLOWISH.

1........	102.85	....	....	....	6........	104.50	95.547	0.65783	91.495
2........	....	96.148	....	....					
3........	109.87	93.018	0.60911	84.722	Average.	105.26	95.498	.64827	90.167
4........	106.23	96.629	.65358	90.911	Max.....	109.87	96.629	.67256	93.540
5........	102.35	96.148	.67256	93.540	Min.....	102.85	93.018	.60911	84.722

ERYTHROSIN.

Number of batches.	Iodin.	Sodium.	$\frac{Na}{I_3}$=(0.090855).		Number of batches.	Iodin.	Sodium.	$\frac{Na}{I_3}$=(0.090855).	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1........	96.915	73.473	0.068873	75.805	8........	96.879	102.09	.095741	105.37
2........	97.051	100.94	.094497	104.00	9........	97.886	103.43	.095995	105.65
3........	99.593	98.277	.089639	98.663					
4........	97.780	97.521	.090604	99.724	Average.	97.952	96.777	.090204	99.146
5........	97.209	....	....	....	Max.....	99.593	103.43	.095995	105.65
6........	96.983	....	....	....	Min.....	96.879	73.473	.068873	75.805
7........	97.035	101.71	.095229	104.81					

Percentages based on theoretical composition—Continued.

INDIGO DISULPHO ACID.

Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}$=(0.71905).		Number of batches.	Sulphur.	Sodium.	$\frac{Na}{S}$=(0.71905).	
			Value.	Per cent.				Value.	Per cent.
	Per cent.	*Per cent.*				*Per cent.*	*Per cent.*		
1........	97.670	103.330	0.71482	99.423	6........	97.818	109.040	.75315	104.730
2........	95.636	98.279	.69430	95.669					
3........	97.600	103.760	.71832	99.910	Average.	97.042	102.149	.71099	98.736
4........	96.148	91.926	.64598	89.848	Max.....	97.818	109.040	.75315	104.750
5........	97.382	106.560	.73934	102.830	Min.....	95.636	91.926	.64598	89.848

ARSENIC DETERMINATIONS ON 86 BATCHES.

During the course of this work it became necessary to determine the exact amount of arsenic contained in 86 of the various batches of certified colors; for this purpose the arsenic method of Seeker and Smith (see p. 212) was devised. These results are expressed in the number of parts of color containing one part of metallic arsenic (As). The numbers in parentheses indicate the number of specimens of the quality indicated.

Naphthol Yellow S (17 specimens).—770,000, 833,000, 1,250,000 (2), 1,428,000 (2), 1,666,000 (3), 2,000,000 (6), 3,333,000 (2).

Ponceau (15 specimens).—588,000, 625,000, 667,000, 714,000 (2), 769,000 (2), 1,000,000 (2), 1,111,000, 1,250,000, 1,666,000 (2), 2,000,000, 2,500,000.

Orange (7 specimens).—200,000, 250,000, 588,000, 1,000,000, 1,111,000, 1,429,000, 5,000,000.

Amaranth (27 specimens).—909,000, 1,000,000, 1,111,000, 1,250,000 (2), 1,438,000 (3), 2,000,000 (4), 2,500,000 (4), 3,333,000 (9), 5,000,000 (2).

Green (8 specimens).—166,000, 200,000, 370,000 (2), 500,000, 666,000, 770,000, 833,000.

Erythrosin (7 specimens).—2,000,000 (2), 5,000,000 (2), 10,000,000 (2), 20,000,000 (1).

Blue (5 specimens).—285,000, 666,000, 1,428,000, 3,333,000 (2).

The United States Pharmacopœia test for arsenic is sensitive to 0.005 mg of arsenious oxid (As_2O_3) which on a sample containing 2 grams of actual or real color would amount to one part of arsenious oxid in 400,000 of color; calculated to metallic arsenic, the basis employed in the foregoing, this would mean 528,000 parts of color for each one part of metallic arsenic.

There are, therefore, in the foregoing 86 lots of certified colors 8 which did not comply with that requirement, namely:

Orange (2 specimens).—200,000 and 250,000.

Green (5 specimens).—166,000; 200,000; 370,000 (2); 500,000.

Blue (1 specimen).—285,000.

The reason for this discrepancy is that in the preparation of the samples for analysis by the United States Pharmacopœia test a loss of arsenic ensued, which is avoided in the Seeker-Smith method now employed in making these determinations. Had the existence of this discrepancy been proven at the time the first analysis was made certification would have been denied to the eight lots above mentioned.

These results further show the position taken early in the work by several of the manufacturers—that a requirement of not more than 1 part of metallic arsenic in not less than 264,000 parts of coal-tar color could not be complied with on a commercial scale—to be untenable; on this basis only 4 out of the 86 lots examined would have been excluded. Further, the position of some manufacturers and dealers that the arsenic requirement ought, for practical manufacturing reasons, not to be more rigorous than one part of metallic arsenic in 26,400 parts of color, or 1 part of arsenious oxid (As_2O_3) per 20,000 parts of color, is not borne out by the data.

SUGGESTED REQUIREMENTS FOR CERTIFIED COLORS.

Although the material embodied in this report gives a very good idea of the composition and quality of substantially 30 different lots of permitted colors prior to the issuance of Food Inspection Decisions Nos. 76 and 77, and of 74 lots of certified colors, yet these data are hardly sufficient to furnish a basis for standards with which each color specimen must comply in detail. The fitness or unsuitability of any lot has been determined by the examination of the analytical data obtained thereon in the Food and Drug Inspection Laboratory at New York; such examination has been applied to the particular color under investigation with respect to its general relationship to the results theretofore achieved. If in some minor quality, as, for example, freedom from salt, the sample was not up to what had been previously accomplished, but in a major quality, as for example, ether extractive, it was equal to or better than what had been previously accomplished, and the pharmacopœial tests for arsenic and heavy metals were satisfied with the exception of iron, and the amount of iron was within the limit previously stated, 0.005 per cent, and the other factors showed a fairly close conformity, such a defect as its salt content would not act as a bar to the passing of the lot; however, no matter how good a color might be in respect to such determinations as ether extractive, if it failed to comply with the United States Pharmacopœia requirement for arsenic or for heavy metals it was not accepted.

These arsenic results have been tabulated to show the distribution of arsenic content (As_2O_3); the numbers at the top are the Green Table numbers; the numbers in the body of the table indicate the number of specimens of the arsenic content stated in the first column; the last column shows the totals of all colors of the arsenic content (As_2O_3) corresponding thereto.

Arsenic content of 86 lots of certified colors.

Arsenic content.	Green Table numbers.							Total.
	4	56	85	107	435	517	692	
1 part in—								
[1] 126,200	......	......	......	......	[1] 1	......	......	1
[1] 151,000	......	......	......	......	[1] 1	......	......	1
[1] 151,500	......	......	[1] 1	......	......	......	......	1
[1] 189,300	......	......	[1] 1	......	......	......	......	1
[1] 215,900	......	......	......	......	......	......	[1] 1	1
[1] 280,300	......	......	......	......	[1] 2	......	......	2
[1] 378,700	......	......	......	......	[1] 1	......	......	1
445,400	......	1	1	......	......	......	......	2
473,500	......	1	......	......	......	......	......	1
504,600	......	......	......	......	1	......	1	2
505,300	......	1	......	......	......	......	......	1
540,900	......	2	......	......	......	......	......	2
582,500	......	2	......	......	......	......	......	2
583,300	1	......	......	......	1	......	......	2
631,000	1	......	......	......	1	......	......	2
688,700	......	......	......	1	......	......	......	1
757,500	......	2	1	1	......	......	......	4
841,600	......	1	1	1	......	......	......	3
946,900	2	1	......	2	......	......	......	5
1,081,000	2	......	......	......	......	......	1	3
1,082,000	......	......	1	......	......	......	......	1
1,089,000	......	......	......	3	......	......	......	3
1,262,000	3	2	......	......	......	......	......	5
1,515,000	6	1	......	4	......	2	......	13
1,893,000	......	1	......	4	......	......	......	5
2,524,000	2	......	......	9	......	......	2	13
3,787,000	......	......	1	2	......	2	......	5
7,157,000	......	......	......	......	......	2	......	2
15,150,000	......	......	......	......	......	1	......	1
	17	15	7	27	8	7	5	86

[1] The certification of these lots was due to an unknown source of error in the analytical method; the analyses made at that time (July, 1909), showed less than 1 part of As_2O_3 per 400,000; see also page 204.

It is therefore somewhat premature to attempt to define rigidly the requirements for composition and purity for colors until a sufficient number of analyses is available to permit a hard-and-fast line to be drawn for each item as required for each color. Until that time the decision as to whether or not a certain color shall be certified must rest with the Department of Agriculture. However, the following requirements are tentatively suggested as being commercially practicable. It should be clearly understood that the tentative requirements here stated are based on the results of actual control, and are not any more searching or numerous than are the requirements for many if not most of the coal-tar dyes or their component parts in the industrial arts, particularly for the various kinds of paint, varnish, and ink making. While it may be that some of the tentative requirements herein defined necessitate the expenditure of considerable work and time, yet that is also true of some of the commercial requirements. Since manufacturers of cheap paints, varnishes, inks, and the like, find it wise and necessary carefully to control the quality of the coal-tar dyes or their component parts which they use, it can not be less wise or necessary to extend the same kind of quality control to those coal-tar dyes intended and sold for human consumption as food. This stand is fully justified by the quality of the coal-tar dyes offered as food colors on the United States markets as described in the foregoing pages. That such control is not only practical, but practicable, is fully proved by the fact that more than 20.5 tons (41,000 pounds) of coal-tar food dyes have been so controlled, examined, and certified under the food inspection decisions hereinbefore mentioned.

The figures are expressed in parts per hundred of actual color contained in the dye and not in parts per hundred of the total substance. The numbers at the head of the columns are the numbers in the Green Tables.

Tentative limits of composition suggested for permitted colors.

(Parts per hundred of actual color.)

Determinations.	Green Tables numbers.						
	4	56	85	107	435	517	692
Insolubles:							
Total	0.070	0.270	0.250	0.130	0.030	0.060	0.450
Nonvolatile on ignition	.040	.150	.050	.050	.010	.020	.250
Ether extractives:							
Neutral	.017	.090	.150	.030	.040	.035	.100
Alkaline	.004	.015	.035	.005	.006	.002	.030
Acid	.050	.017	.060	.010	.008	....	.150
Common salt	.600	5.000	2.000	2.500	.600	.400	3.000

In addition to these there are the following requirements applicable to all colors:

1. The absence of admixed dye must be convincingly demonstrated by suitable test.

2. *Arsenic.*—Test 17 of the United States Pharmacopœia, 1900, applied to so much of the specimen as represents 3.5 grams of actual dye must give a negative response. Such negative result must be reenforced by a check test identical with the test on the dye with the addition of 0.005 mg of arsenic (As_2O_3) to the dye prior to treatment, and this check test must produce a positive result for the presence of arsenic. Ignitions in the preparation of the material for the test must be made in porcelain.

Any other mode of testing which is demonstrated to be capable of detecting 0.005 mg added arsenic (As_2O_3) in so much of the specimen as represents 3.5 grams of actual color will, of course, be accepted. This quantity, however, is only tolerated tentatively pending further investigations relative to the complete, or practically complete, elimination of arsenic from foods, especially those which are largely used by children, such as candies.

3. *Heavy metals.*—Test 121 of the United States Pharmacopœia as revised May 1, 1907, using so much of the specimen as represents one part of actual dye must give a negative response for all metals except iron, which may be present in amounts not in excess of 0.005 per cent of iron based on the dye actually present in the specimen.

4. None of the dyes offered for certification shall contain any Glauber's salt or sodium sulphate in any form, nor shall they contain any added sugar, dextrin, or other loader, filler, or reducer for any purpose whatsoever, and convincing proof of the absence of any or all of them must be submitted.

5. The ether extractives are to be made successively upon water solutions of the dye, first neutral, then made alkaline with caustic soda, and then made acid with hydrochloric acid, using washed or sodium, dried ether.

6. The sulphur content of the sulphur-containing dyes must agree substantially with the theoretical; likewise the sulphated ash figures of all must agree substantially with the theoretical; variations between these two sets of figures, as long as they are consistent with each other, will not be reason for rejection.

7. In the case of No. 4 proof must be submitted showing that the specimen is of the sodium or potassium variety, and if it is a mixture of these two varieties the proportion of each present in the mixture must be stated.

8. In the case of No. 56 the crude cumidin employed may have a boiling point of from 220° to 230° C., and may be liquid or solid; the absence of any compound of S or G salt must be convincingly shown.

9. In the case of No. 85 convincing proof must be submitted that beta-naphthol orange if present at all is present in an amount not in excess of 5 per cent of the coal-tar dye present.

10. In the case of No. 107 the absence of any compound of S or G salt must be convincingly shown.

11. In the case of No. 435 the product should be free from calcium; convincing proof of absence of No. 434 must be submitted.

12. In the case of No. 517 the actual dye must contain not less than 56 per cent of iodin (sodium basis) and must not contain any other halogen; the kind and amount of metallic base, whether sodium, potassium, or the like, must be shown.

13. In the case of No. 692 the absence of indigo monosulphonic acid and of nonsulphonated indigo must be convincingly shown.

14. Each foundation certificate must be filed in duplicate, but need not be executed in duplicate and must contain a summarized or tabulated statement of all the quantitive results contained in the certificate, also a tabulation or summary of the qualitative tests made, together with the results of such tests, all stated on one sheet, so that the certificate will bear within itself its own summary and conclusions.

15. The fundamental analytical data must be given with such fullness as to permit efficient checking of the calculations, and the arithmetical operations performed should be indicated wherever needful to avoid confusion, or to facilitate the work of the checking chemist, or make the meaning of the certificate more plain.

There are freely quoted in the United States market two substances, paranitranilin and betanaphthol, which are subject to much competition, the prices for which, wholesale, are not far from 25 cents and 9 cents, respectively. A specimen of each has been examined, and, as before stated, they have both been found to be of such purity, with respect to arsenic, heavy metals, and general cleanliness, that had they been capable of use in food products, no objection against their use on this score could reasonably be raised; certainly no such objection could be successfully maintained.

The following table discloses the chemicals entering into the manufacture of paranitranilin and of betanaphthol, as well as of each of the seven permitted colors; the ingredients numbered 1 to 7 are used in the manufacture of these two substances, as well as in the seven permitted colors, as indicated by the "x" entries; ingredients 8 to 20 are used only in the manufacture of the seven permitted colors, and not in paranitranilin and betanaphthol.

Comparison of chemicals entering into the composition of the seven permitted colors and of paranitranilin and betanaphthol.

Determinations.	4. Naphthol Yellow S.	56. Ponceau 3R.	85. Orange I.	107. Amaranth.	435. Light Green.	517. Erythrosin.	692. Indigo disulpho acid.	Paranitraniline.	Betanaphthol.
1. Naphthalene	x	x	x	x	....	x	x	....	x
2. Benzol	....	x	x	....	x	x	x	x	....
3. Sulphuric acid	x	x	x	x	x	x	x	x	x
4. Nitric acid	x	x	x	x	x	....	....	x	....
5. Metallic iron	....	x	x	x	x	....	....	x	....
6. Acetic acid	....	x	x	x	x	....	x	x	....
7. Caustic soda or potash	x	x	x	x	....	x	x	....	x
8. Carbonate of soda	x	x	x	x	x	....	x	....	....
9. Common salt	x	x	x	x	....	x	x	....	....
10. Wood or methyl alcohol	....	x	....	....	....	x	....	....	....
11. Lime	....	x	....	x	x	....	x	....	....
12. Hydrochloric acid	....	x	x	x	....	....	....	....	....
13. Sodium nitrite	....	x	x	x	....	....	....	....	....
14. Ethyl chlorid	....	....	....	....	x	....	....	....	....
15. Peroxid of lead or of manganese	....	....	....	....	x	....	....	....	....
16. Phosgene	....	....	....	....	x	....	....	....	....
17. Iodin	....	....	....	....	....	x	....	....	....
18. Metallic mercury	....	....	....	....	....	x	x	....	....
19. Ferric chlorid	....	....	....	....	....	x	....	....	....
20. Chlorin	....	....	....	....	....	....	x	....	....
	6	13	11	11	10	9	10	5	3

With respect to the arsenic content of the finished product, it can be asserted, without any fear whatever of successful contradiction, that the arsenic finds its way into the goods by way of the sulphuric acid which is used in the manufacture of all the permitted colors, as well as in the manufacture of Paranitranilin and Betanaphthol. If arsenic from this source can be kept out of these substances it can also be excluded from the permitted colors.

Turning now to those ingredients below the parallel lines, the only means of introducing arsenic would be through the hydrochloric acid used, which in turn derives its arsenic from the sulphuric acid used in its manufacture, and since arsenic free-sulphuric acid can be used in the first stages of producing the seven permitted colors and the paranitranilin and betanaphthol, it can also be employed in the manufacture of the hydrochloric acid used in the subsequent stages of manufacture of the seven permitted colors. Therefore, it seems unreasonable to permit a higher arsenic content in food colors than in paranitranilin and betanaphthol, which are sold in open competition, and which are used for the production of the very cheapest colored cloths, inks, and paints.

With reference to the content of iron, and other heavy metals, which satisfied the pharmacopœial tests in the case of paranitranilin and betanaphthol, these materials probably enter the product from the vessels in which the manufacturing operations are performed. The same kind of vessel used in the manufacture of paranitranilin and betanaphthol is used in making the seven permitted colors, and since the heavy metals can be and are kept out of these two substances to the extent required by the Pharmacopœia, there seems to be no good reason why they should not be kept out of the seven permitted colors when made for use in food products.

Since all these defects in the seven permitted colors can be obviated in their first stage of manufacture (as is shown in the case of paranitranilin and betanaphthol, and in some actual commercial samples of 1907, see sections XIV and XV), they are commercially avoidable at subsequent stages of manufacture, and there is no good reason why they should be then introduced.

XVII. METHODS OF ANALYSIS USED IN TESTING COLORS FOR CERTIFICATION.

INTRODUCTION.

The exact analytical methods developed and tested in the New York Food and Drug Inspection Laboratory and used in obtaining the analytical data contained in the preceding chapter are described in the following pages. These descriptions were written by A. F. Seeker, of that laboratory, under whose immediate supervision all the laboratory work was done, and they represent a great deal of work, extending over more than three years. The methods are submitted in the hope and expectation that in their wider application by a larger number of chemists any defects in the methods or conclusions drawn from the results will be detected and rectified. Experience in the New York laboratory has shown that even different chemists of varying degrees of experience in this particular line of work obtain concordant duplicates after a comparatively short laboratory acquaintance with these methods.

The identity of these colors and their freedom from foreign dyes is shown by the close agreement of their elements, as determined by analysis, with their theoretical composition; their behavoir toward reagents as given by standard works on dyes; their distribution between the layers when neutral, alkaline, and acid aqueous solutions are shaken with various immiscible solvents; uniformity of shade in the spots produced by particles of the dry color blown over the surface of wet filter paper, or water, and over concentrated sulphuric acid; uniformity of shade produced by a 0.5 per cent dyeing on wool under standard conditions, with similar dyeings from fractions produced by partial precipitation, by partial salting out, by fractional crystallization, and by extraction with alcohol or some liquid in which the pure color is not very soluble; and the behavior of the dye in acid and alkaline solution toward cotton. Which of these tests are needed to prove conclusively the identity of certain dyes or to establish their absence, is a matter that varies so much from case to case that it must be left largely to the individual judgment to decide on the best combination of tests, and for that reason such combinations are not here offered. The general methods and procedures just outlined have, however, when properly combined, led to satisfactory results.

In order to compare the results of the color analyses on the same basis, the actual figures obtained in the various determinations besides being reported as found, are also recalculated on a basis of 100 parts of actual color, i. e., the sum of the percentages of material which is not coloring matter, such as moisture, total insoluble matter, sodium chlorid, sodium sulphate, and ether extractives is deducted from 100 and the percentages of the various constituents found divided by the difference, the quotient being then multiplied by 100. The percentage of sodium in actual dye is calculated from the sulphated ash. The methods of analysis of the seven permitted colors of Food Inspection Decision No. 76 are given in the following order:

1. Naphthol Yellow S.
2. Ponceau 3R.
3. Orange I.
4. Amaranth.
5. Light Green S F Yellowish.
6. Erythrosin.
7. Indigo Disulphoacid.

NAPHTHOL YELLOW S.

MOISTURE.

Dry from 1 to 2 grams of the finely powdered dye in an air oven at 120° to 125° C. to constant weight.

TOTAL INSOLUBLE MATTER.

Dissolve 5 grams of color in 200 cc of hot distilled water, filter through a tared gooch, wash till the washings run through colorless, dry the insoluble residue at 105° C., and weigh.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Ignite the gooch containing the insoluble matter of the last determination at a low red heat, cool, and weigh.

SODIUM CHLORID.

Dissolve 3 to 5 grams of dye in 200 cc of water, acidify the solution with nitric acid, and precipitate the chlorin as silver chlorid. The latter is separated, washed, ignited, and weighed in a tared gooch crucible in the usual way.

SODIUM SULPHATE.

Dissolve 1 gram of dye in about 100 cc of water in a 200-cc graduated flask, add 40 cc of a 20 per cent solution of potassium chlorid, shake the mixture well, make up to mark with water, shake again, and then filter through dry paper. Treat 100 cc of the filtrate (representing 0.5 gram of dye) with 5 cc of 10 per cent barium chlorid solution without acidifying, and allow to stand overnight. If a precipitate forms, it is filtered, washed, ignited, and weighed in the usual way.

HEAVY METALS.

Treat the sulphated ash from 1 gram of the sample with 20 cc of water, digest with 10 cc of 10 per cent hydrochloric acid till solution is complete, place 3 cc of the mixture in a test tube, add 10 cc of freshly prepared hydrogen sulphid test solution (U. S. P.), shake the mixture, warm to 50° C., stopper, and allow to stand in a warm place (at about 35° C.) for half an hour. Run a blank test at the same time, with the same amount of hydrogen sulphid solution, using water instead of the solution containing the color ash. No turbidity other than that sometimes produced by slight separation of sulphur should appear in this test. Both tubes are then made slightly alkaline with ammonium hydroxid, and no precipitate should be produced, although a slight coloration, due to the presence of a small amount of iron, sometimes occurs. If this coloration is very marked the amount of iron should be determined. This is done by digesting the sulphated ash from a weighed amount of the sample with hydrochloric acid until all of the iron has gone into solution. The solution is filtered, and the filtrate poured into an excess of hot, pure, freshly prepared sodium hydroxid (by sodium) solution in a platinum dish. The precipitate is washed, dissolved in dilute hydrochloric acid, and again precipitated with ammonium hydroxid. The last precipitate is washed, ignited, and weighed in the usual manner.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 10 grams of the dye in 200 cc of water, heating to insure complete solution of the color, add about 10 cc of strong bromin water to convert any arsenite to arsenate. Make the mixture alkaline with a few cubic centimeters of strong ammonium hydroxid. Twenty cubic centimeters of a sodium phosphate solution containing 100 grams of crystallized sodium phosphate per liter are next added from a pipette, after which magnesia mixture (containing 55 grams of hydrated magnesium chlorid, 55 grams of ammonium chlorid, and 88 cc of ammonium hydroxid, specific gravity 0.9 per liter) is added from a burette, stirring vigorously. The amount of magnesia added should be in slight excess of that necessary completely to precipitate the phosphate and should previously be ascertained by blank experiment. Then add 10 cc of ammonium hydroxid (specific gravity 0.90), and allow the whole to stand for at least three hours; separate the precipitate by filtration and wash it free, or nearly so, of dye with ammonium hydroxid containing one-tenth its volume of ammonia (specific gravity 0.90). Dissolve the precipitate from the paper with 1:1 nitric acid, the washings being collected in a large porcelain crucible; add 5 cc of sulphuric acid to the contents of the crucible, and evaporate the whole almost to dryness. It is not necessary that the solution should be colorless at this point, a brown colored solution giving equally accurate results. Add 20 cc of water to the residue in the crucible and then 10 cc of a saturated solution of sulphur dioxid; evaporate the solution to a sirupy consistency to remove the sulphur dioxid, and then take up in 20 cc of water and place in a 30-cc evolution bottle, add 5 cc of concentrated sulphuric acid, and determine the arsenic in the form of apparatus (fig. 1) used by Bishop in his modification of the Gutzeit test, the stains obtained being compared with those given by known amounts of arsenic.

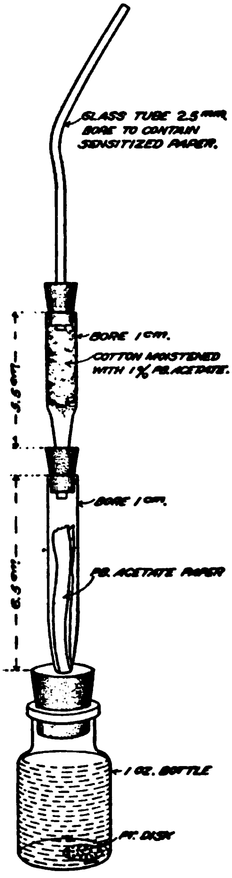
GLASS TUBE 2.5 mm BORE TO CONTAIN SENSITIZED PAPER.
BORE 1 cm.
COTTON MOISTENED WITH 1% PB. ACETATE.
5.5 cm
BORE 1 cm.
PB. ACETATE PAPER
6.5 cm
1 OZ. BOTTLE
PT. DISK

Fig. 1.—Apparatus for the determination of arsenic.

The apparatus used by Bishop consists of the following parts: A 30-cc salt mouth evolution bottle into which is fitted a one-hole rubber stopper carrying a glass tube 6.5 cm long with an internal diameter of 1 cm, this tube in turn being provided with a one-hole rubber stopper fitted with another tube of the same diameter and 5.5 cm long, the diameters of both tubes being constricted at the points at which they are inserted in the rubber stoppers. A third glass tube 15 cm long, having an internal diameter of 2.5 mm, is fitted into the second at its upper end by means of a rubber stopper. The first tube contains a strip of filter paper which has been saturated with a 5 per cent solution of lead acetate and dried. The second tube contains a loosely packed plug of cotton-wool freshly moistened with a 1 per cent solution of lead acetate. Into the topmost tube is inserted the strip of sensitized paper to receive the arsenic stain. The arsin is generated by introducing into the evolution bottle six pieces of Kahlbaum's stick zinc (arsenic-free for forensic purposes) weighing in all about 8 grams, and to assist in an active and constant evolution of gas a disk of platinum is also placed in each bottle to form an electrolytic couple. The evolution of gas is allowed to proceed for one hour. The stains are produced on strips of hard pressed white paper (2 mm wide and 120 mm long) that has been sensitized by being dipped in a 5 per cent alcoholic solution of mercuric chlorid and then dried. (NOTE.—It has recently been found that mercuric bromid yields stains that are more evenly distributed and also produces standards that are incomparably more permanent.) For purposes of comparison it is better not to develop the strips stained by the arsin, though some prefer to dip the stains in ammonium hydroxid, which causes them to become black. A blank is run with each set of determinations.

ETHER EXTRACTIVES.

Dissolve 10 grams of color in 150 cc of water and extract in a separatory funnel with ether that has been washed with water (using three 150 cc portions of water for each liter of ether). Extract the color solution with two 100 cc portions of this ether, shaking thoroughly for one minute, and wash the combined ether extract successively with 35, 20, and 10 cc of water made alkaline or acid, as the case requires, with 1 cc of tenth normal alkali or acid per 100 cc of water. Decant the ether from the mouth of the separatory and rinse the funnel once with 5 cc ether. The color solution is first extracted neutral, the extracted solution being then rendered alkaline with 2 cc of a 10 per cent solution of caustic soda and again extracted with two 100-cc portions of ether. In acidifying for the third extraction, add twice the amount of hydrochloric acid (1 to 3) necessary to neutralize the alkali, and repeat the extraction with two 100-cc portions of ether. Place the neutral, alkaline, and acid extracts in a dust-free atmosphere, and allow the ether to evaporate spontaneously, after which dry the residues to constant weigh over sulphuric acid, using flat-bottomed dishes 2¾ inches in diameter, 1¼ inches in height, and of about 100 cc capacity. The dishes should be thoroughly cleaned, wiped dry, and allowed to stand in a sulphuric acid desiccator at least two hours before weighing. In order to avoid the generation of static charges of electricity, they should not be wiped immediately before weighing. Run two blank determinations with each series of ether extracts, and deduct the average gain in weight of these two blanks from the weights obtained in the other determinations.

SULPHATED ASH.

Weigh accurately 0.5 to 1 gram of the color into a wide platinum dish, moisten with concentrated sulphuric acid, and ignite cautiously, avoiding spattering; moisten the residue repeatedly with sulphuric acid and ignite until all the carbon is removed and a white or reddish ash is obtained. This is finally ignited at a fairly bright red heat, cooled, and weighed. The aqueous solution of this ash should be neutral to litmus, and may be used in a quantitative test for potassium.

CALCIUM.

Digest the residue from the sulphated ash determination with hydrochloric acid, render the solution alkaline with ammonium hydroxid, filter, and precipitate the calcium in the filtrate with ammonium oxalate. The precipitate of calcium oxalate is filtered on a tared gooch, washed, dried at 100° C. and weighed as calcium oxalate, the calcium being calculated from the formula $CaC_2O_4.H_2O$. This method is applicable only in cases where the amount of calcium is very small. When the amount exceeds 0.5 per cent it should be determined by digesting the sulphated ash with ammonium sulphate solution made acid with hydrochloric acid, and precipitating the iron by an excess of ammonium hydroxid. The precipitate is washed, dissolved in hydrochloric acid, and reprecipitated, the filtrate and washings being added to those obtained from the first precipitate. The calcium is precipitated in the combined filtrates with ammonium oxalate, the oxalate being filtered, washed, ignited, and weighed in the usual way; the residue is weighed as oxid after ignition to bright redness.

SULPHUR.

Determine upon 0.2 to 0.3 gram portions by the Carius method (Gattermann, Practical Methods of Organic Chemistry, 1901, p. 81), using 3 cc of fuming nitric acid (sp. gr. 1.5), and heating the sealed tubes to 300° C. for at least eight hours.

NITROGEN.

Use the method of Dumas (Gattermann, Practical Methods of Organic Chemistry, 1901, p. 85).

PONCEAU 3R.

MOISTURE.

Dry 1 to 2 grams of the finely ground dye at 109° to 110° C. in a current of dry hydrogen to constant weight.

TOTAL INSOLUBLE MATTER.

Determine as under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as under Naphthol Yellow S, page 211.

SODIUM CHLORID (SEEKER AND MATHEWSON'S METHOD).

Mix 2 grams of dye thoroughly with from 2 to 3 grams of sodium carbonate, moisten with water to form a paste, again mix, dry, and ignite at a low red heat. By moistening, drying, and reigniting a more complete destruction of organic matter is obtained. Break up the charred mass, introduce into a 200-cc graduated flask with about 100 to 150 cc of hot water, and add an excess of potassium permanganate to oxidize sulphids. Destroy the excess of permanganate by adding sulphur dioxid solution until the red color changes to brown, then cool the mixture and make up to the mark with water. Filter through a dry paper, acidify 100 cc of the filtrate with nitric acid, and precipitate the chlorin as silver chlorid by the addition of silver nitrate. If the solution should be brownish from a trace of organic matter the silver chlorid does not readily coagulate and tends to pass through the filter. In this case a few drops of potassium permanganate solution are added to this acid mixture, the organic matter being almost instantly oxidized. The mixture is then decolorized with a few drops of sulphur dioxid solution, the silver chlorid is separated on a tared gooch, washed, ignited, and weighed in the usual manner.

The following method may also be used for Ponceau 3R:

Dissolve 5 grams of the dye in 15C cc hot water, wash into a 250-cc graduated flask, and add 25 cc of a 10 per cent solution of barium nitrate. Cool the mixture, make up to the mark, and filter through a dry paper; acidify 100 cc of the filtrate, representing 2 grams of color, with nitric acid, and treat with silver nitrate solution, the precipitated silver chlorid being separated, washed, ignited, and weighed in a tared gooch crucible in the usual way.

SODIUM SULPHATE.

Dissolve 2 grams of dye in 100 cc of hot water, wash into a 200-cc graduated flask, add 50 grams of pure sodium chlorid, cool, and make up to the mark with water. Filter through a dry filter, dilute 100 cc of the filtrate to 300 cc, acidify with hydrochloric acid and precipitate the sulphates with barium chlorid. Filter, wash, ignite, and weigh in a tared platinum gooch in the usual way.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN (SEEKER AND MATHEWSON'S METHOD).

Treat 2 grams of the color with 25 cc of a saturated solution of sulphur dioxid and 1 gram of zinc dust and warm the mixture gently until it becomes colorless. This should take place in from two to three minutes, but if it does not add more sulphur dioxid solution in small portions at a time until the color is destroyed. Then add 30 cc of concentrated sulphuric acid and 0.7 gram of mercuric oxid or its equivalent of metallic mercury and digest the mixture, make alkaline, and distil as directed on page 6, Bulletin 107, Revised, Bureau of Chemistry, United States Department of Agriculture, under the Kjeldahl process.

CRUDE CUMIDIN.

Dissolve 20 grams of dye in 400 cc of hot water and pour the solution, a little at a time, into a reducing solution composed of 75 grams of stannous chlorid dissolved in 180 cc of concentrated hydrochloric acid. Heat the mixture on a steam bath until it is straw colored, cool, add an excess of sodium hydroxid, and extract in a separatory funnel with ether. Separate the ether layer and distil off the solvent until the residue measures about 50 cc. Then cautiously heat over the steam with constant agitation until the odor of ether disappears, after which the last of the moisture is removed by introducing a few pieces of solid caustic soda and allowing to stand. The residue consists of crude cumidin and should boil above 220° C. Cumidin nitrate is sparingly soluble in water.

ORANGE I.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 1 gram of dye in 100 cc water contained in a 200-cc graduated flask and treat the solution with 60 cc of a 20 per cent solution of potassium chlorid. Make the mixture up to the mark with water, shake, filter through a dry paper, dilute an aliquot of 100 cc of the filtrate to 200 cc, acidify with 1 cc of 10 per cent hydrochloric acid, treat with 5 cc of 10 per cent barium chlorid solution, and allow to stand over night. If a precipitate has been formed this is separated, ignited, and weighed in the usual way.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 2 grams of dye in a mixture of 130 cc water and 70 cc of 95 per cent alcohol. Add about 10 cc of strong bromin water to convert any arsenite to arsenate. Make the mixture alkaline with a few cubic centimeters of strong ammonium hydroxid and add, from a pipette, 20 cc of a sodium phosphate solution containing 100 grams of crystallized sodium phosphate per liter, after which magnesia mixture (containing 55 grams of hydrated magnesium chlorid, 55 grams of ammonium chlorid, and 88 cc of ammonium hydroxid, sp. gr. 0.9, per liter) is added from a burette, stirring vigorously. The amount of magnesia mixture to be added should be in slight excess of that necessary to precipitate the phosphate completely, and should be previously ascertained by a blank experiment. Finally add 10 cc of ammonium hydroxid (sp. gr. 0.96) and allow the whole to stand for at least eight hours. Separate the precipitate by filtration and wash it free, or nearly so, of dye with a mixture of one-third alcohol and two-thirds water containing one-tenth its volume of ammonium hydroxid (sp. gr. 0.90). Dissolve the precipitate from the paper with 20 per cent sulphuric acid, the washings being collected in a large porcelain crucible. Add 5 cc of concentrated nitric acid to the contents of the crucible and evaporate the whole almost to dryness. The mixture need not be colorless at this point, a brown colored solution giving equally accurate results. Add 20 cc of water to the residue in the crucible and then 10 cc of a saturated solution of sulphur dioxid. Evaporate the solution to a syrupy consistency to remove sulphur dioxid, and then take up in 20 cc of water, place in a 30-cc evolution bottle, add 5 cc of concentrated sulphuric acid, and determine the arsenic by the modified Gutzeit method as given under Naphthol Yellow S, page 213.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Ponceau 3R, page 217.

TEST FOR ORANGE II (SMITH AND MATHEWSON'S METHOD).

The following solutions are required:

(1) Fifteen per cent titanium trichlorid.

(2) Freshly prepared diazotized sulphanilic acid. Heat a mixture composed of 1 gram of sulphanilic acid, 10 cc of water, and 20 cc of concentrated hydrochloric acid on a steam bath for five minutes with occasional shaking. Cool to about 10° C. and add slowly 10 cc of a 1 per cent solution of sodium nitrite. Allow the mixture to stand at about 10° for 10 minutes, dilute to 1 liter, and shake until all the solid particles have dissolved.

(3) Stannous chlorid, prepared by dissolving 40 grams of stannous chlorid in 100 cc of concentrated hydrochloric acid. Dilute this ten times with water, immediately before using.

Place 2 cc of a 0.1 per cent solution of the dye in a colorimeter tube having a capacity of about 100 cc, add a small drop of titanium trichlorid solution and shake until the mixture is decolorized. Standards containing the same quantity of color composed of a mixture of pure Orange I and known amounts of Orange II are treated in the same way, the volume of the solution at this point to measure less than 5 cc. Dilute the decolorized solutions to 50 cc with 95 per cent alcohol and equalize the temperature by immersing the tubes in water at room temperature. (Note.—A slight coloration that may develop at this point may be disregarded.) Allow the tubes to stand in the water for about five minutes, add 2 cc of the diazotized sulphanilic acid, and mix thoroughly. If the titanium trichlorid has not been used in too great excess, the first few drops of the diazotized sulphanilic acid will cause a formation of color. Allow the coupling to proceed for three minutes and then add 5 cc of the diluted stannous chlorid with vigorous shaking. In two minutes the blue color due to Orange I will disappear, leaving only the pink caused by the coupling product of Orange II. The depth of color in the tube containing the dye under examination may then be compared to the standards.

AMARANTH.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 2 grams of dye in 100 cc of warm water in a 200-cc graduated flask, and add 36 grams of pure sodium chlorid. Allow the mixture to stand with frequent shaking for one hour, and after cooling make up to the mark with a saturated salt solution. Shake the mixture and filter through a dry paper; dilute 100 cc of the filtrate (representing 1 gram) with water, acidify with hydrochloric acid, and precipitate the sulphates with barium chlorid. The precipitate is separated, washed, and ignited upon a tared platinum gooch crucible.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Ponceau 3R, page 217.

LIGHT GREEN S F YELLOWISH.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 5 grams of the color in 100 cc of water, warming by means of a gentle heat. Dissolve 11 grams of safranin in a separate portion of 400 cc of water, also by warming, taking care in both instances to prevent loss by evaporation. Mix the two solutions, shake thoroughly, and filter through a dry filter. Render an aliquot portion of the filtrate alkaline with sodium hydroxid and remove the excess of safranin by shaking with two successive portions of amyl alcohol. Wash the combined amyl alcohol layers with two portions of water and add the washings to the main aqueous solution, which is then acidified with hydrochloric acid and sulphates determined in the usual manner by preciptiation with barium chlorid as barium sulphate.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC.

Determine as given under Naphthol Yellow S, page 212.

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine on 2-gram portions by Gunning's modification of the Kjeldahl process, using a little copper sulphate to assist the oxidation (see page 7, Bulletin 107, Revised, Bureau of Chemistry, United States Department of Agriculture).

ERYTHROSIN.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Dissolve 5 grams of the dye in 400 cc water and acidify with dilute nitric acid. Make the mixture up to 500 cc with water, and then filter through a dry filter. Determine chlorids in an aliquot of 200 cc of the filtrate by precipitation with silver nitrate, washing, igniting, and weighing the silver chlorid in a tared gooch crucible in the usual manner.

SODIUM SULPHATE.

Employ another aliquot of the filtrate obtained after precipitating the color acid as above in the determination of sulphates, precipitating the latter as barium sulphate in the usual manner.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Dissolve 16 grams of dye in 370 cc of water, add 5 cc of strong bromin water, and finally 25 cc of dilute sulphuric acid (1 to 4). Shake thoroughly and filter through a dry filter. Place an aliquot of 250 cc, representing 10 grams of color from the filtrate, in a porcelain casserole, add 5 cc concentrated nitric acid (very important to prevent loss of arsenic), and evaporate till fuming has ceased. Reduce the residue with sulphur dioxid solution, evaporate to small bulk, and determine the arsenic in the form of apparatus used by Bishop in his modification of the Gutzeit test. See under Naphthol Yellow S, pages 212 and 213. (NOTE.—It is somewhat difficult at times to recover 250 cc of filtrate, but less may be used and a correction made, if necessary.)

ETHER EXTRACTIVES.

Determine as given under Naphthol Yellow S, page 214, omitting the acid extraction.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

IODIN, BROMIN, AND CHLORIN (CORNELISON'S METHOD).

Mix 0.2 to 0.3 gram of the sample with 2 grams of pure potassium bichromate and 15 cc of strong sulphuric acid in the evolution flask of an apparatus made entirely of glass, with ground-glass joints. Thoroughly mix the contents of the evolution flask, so that all lumps are disintegrated, and then heat at 100° C. for 15 minutes, after which raise the temperature to 150° C. for thirty minutes, a current of air dried over calcium chlorid and potassium hydroxid being drawn through the apparatus during this time. Iodin remains in the evolution flask as iodic acid; bromin passes off as such, and may be absorbed by allowing the air passing through the apparatus to bubble through 1 per cent sodium hydroxid. Chlorin passes out of the evolution flask as chromyl chlorid, and may also be absorbed in sodium hydroxid. Cool the mixture containing the iodic acid, and reduce the chromic acid by addition of sulphur dioxid, about 20 cc of a saturated solution being required. When enough has been added, the precipitated iodin redissolves, and the clear green color of chrome alum appears. Filter, wash the paper with distilled water, dilute the filtrate and washings to about 300 cc, and add an excess of silver nitrate. Boil till the silver iodid has flocculated, allow to stand for a few hours, and separate and weigh the silver iodid in a tared gooch.

It sometimes happens that the mixture containing the iodic acid, after the reduction with sulphur dioxid, becomes turbid, owing, apparently, to separation of a basic chromium sulphate. Very often the turbidity can not be removed by filtering, and it has been found advisable in this case to reject the determination and begin anew.

IODIN (SEEKER AND MATHEWSON'S METHOD).[1]

Place from 0.3 to 0.4 gram of the erythrosin in a porcelain casserol, dissolve this in 5 cc of a 10 per cent sodium hydroxid solution, then add 35 cc of a 7 per cent solution of potassium permanganate. After mixing, cover the vessel with a watch crystal, and add 10 cc of nitric acid, keeping the dish covered. Agitate the mixture, place on a steam bath, and keep covered until spattering ceases, after which remove the watch glass and allow evaporation to proceed to dryness.[1] Treat the residue with 5 cc of 7 per cent potassium permanganate and 5 cc of concentrated nitric acid and again evaporate to dryness. Then add about 50 cc of water and 5 cc of concentrated nitric acid to the residue, following this by 40 cc of a saturated solution of sulphur dioxid, and allow the whole to stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxid of manganese has dissolved. Filter, and wash the paper with water. Add an excess of silver nitrate to the filtrate, and boil until sulphur dioxid has been expelled and the silver iodid has flocculated. Separate, wash, and weigh the precipitate in the usual manner.

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[1] U. S. Dept. Agr., Bureau of Chemistry Circular 65. The estimation of iodin in organic compounds and its separation from other halogens.

TOTAL HALOGENS.

Mix 0.5 to 1 gram of the dye with 4 grams of potassium carbonate, moisten to a paste, again thoroughly mix, cover with a layer of dry potassium carbonate, dry, and ignite at a low red heat. Break up the char thoroughly, digest with about 200 cc water, and filter. Wash the insoluble matter until the washings no longer react with silver nitrate; then acidify the filter and washings with nitric acid and precipitate the halogens in the usual way as silver salts.

INDIGO DISULPHOACID.

MOISTURE.

Determine as given under Ponceau 3R, page 215.

TOTAL INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

NONVOLATILE OR INORGANIC INSOLUBLE MATTER.

Determine as given under Naphthol Yellow S, page 211.

SODIUM CHLORID.

Determine as given under Ponceau 3R, page 215.

SODIUM SULPHATE.

Dissolve 2 grams of the dye in about 160 cc of water and treat the solution with 40 grams of pure sodium chlorid. After the salt has dissolved, make up the volume to exactly 200 cc. Shake the mixture thoroughly and filter through a dry filter. Dilute 50 cc of the filtrate with 200 cc of water, acidify with 1 cc of 10 per cent hydrochloric acid, and treat with an excess of barium chlorid solution. After standing overnight, the precipitate is separated, washed, ignited, and weighed in the usual way.

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[1] In the operation of drying particular care should be observed to prevent access of reducing gases to the mixture.

HEAVY METALS.

Determine as given under Naphthol Yellow S, page 212.

ARSENIC (SEEKER AND SMITH'S METHOD).

Treat 10 grams of dye in a Kjeldahl flask with 100 cc water and 10 cc concentrated nitric acid. Warm gently and finally boil until all action has ceased. Transfer to a beaker, make alkaline with ammonium hydroxid, and proceed from this point, as in the case of the solution of Naphthol Yellow S, page 212, continuing from the point at which sodium phosphate is added.

ETHER EXTRACTIVES.

Dissolve 3 grams of dye in 200 cc of water and extract with ether, as directed under Naphthol Yellow S, page 214.

SULPHATED ASH.

Determine as given under Naphthol Yellow S, page 214.

CALCIUM.

Determine as given under Naphthol Yellow S, page 215.

SULPHUR.

Determine as given under Naphthol Yellow S, page 215.

NITROGEN.

Determine as given under Light Green SF Yellowish, page 222.

XVIII. ADDENDA.

ADDITIONAL EXAMINATION OF COAL-TAR DYES.

Since the foregoing report was written the chemical examination of the coal-tar dye specimens collected in the summer of 1907 has been completed. This investigation shows that 7 of the Green Table numbers then reported were not on the market, although those furnishing the specimens included them at the time that the samples were supplied. These numbers are as follows: *not physiologically examined,*

G. T. 49, 60, 104, 518, 523; *physiologically examined*, with unfavorable result, G. T. 516; with favorable result, G. T. 520.

The chemical examination also shows that each of 9 additional Green Table numbers occurred once among the unreported numbers. They are as follows: *not examined physiologically*, G. T. 20, 328; *examined physiologically:* with unfavorable results, G. T. 602; with favorable results, G. T. 92, 477, 521; with contradictory results, G. T. 16, 43, 163. G. T. 20 is anilin azo (1:8) dioxy naphthalene (3:6) disulpho acid. G. T. 328 is diamino-stilbene-disulpho acid-disazo-phenol.

This chemical examination finally shows that beside the Green Table numbers there occurred, once each, the following: sulphonated Victoria Blue B and a sulphonated rhodulin and twice, amido-azo-toluene-azo-alpha-naphthol.

It is clear that this revised statement of facts is without influence on the 7 permitted dyes selected; its only effect is to add G. T. 92 (yellow), 477 (blue), and 521 (red) (each occurring but once) to, and to subtract 520 (red) (said to occur twice) from, the 16 dyes (page 166) from among which the 7 permitted dyes were selected, thus giving 18 instead of 16 dyes to choose from; however, the final selection is wholly unaffected thereby. It is also clear that any and all intermediate conclusions or comparisons are only slightly and immaterially affected, if at all, by the results of this additional examination.

SUPPLEMENTARY LIST OF TRADE NAMES OF COAL-TAR COLORS.[1]

Acid Yellow S (4).
Alkali Blue (477).
Azo Acid Rubin 2B (107).
Azo Yellow (92).
Crocein Scarlet O extra (164).
Dimethylanilin Orange (87).
Eosin, water soluble (512).
Fast Red A (102).
Fast Red B (65).
Fast Red E (105).
Fast Yellow Y (8).
Indian Yellow (92).
Indulin R and B (601).
Leucindophenol (572).
Methyl Orange (87).
Naphthylamin Pink (614).
Naphthylene Blue R in crystals (639).
Soluble Blue 8B and 10B (479).
Sulphur Yellow (4).
Thiochromogen (659).
Victoria Blue (488).
Wool Black (166).
Yellow T (84).

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[1] See also page 148.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 148.

H. W. WILEY, Chief of Bureau.

THE TOXICITY OF CAFFEIN:

AN EXPERIMENTAL STUDY

ON DIFFERENT SPECIES OF ANIMALS.

BY

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Chief Pharmacological Laboratory, Division of Drugs,

AND

J. B. RIEGER,
Assistant Chemist.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., November 14, 1911.

Sir: I have the honor to submit for your approval a manuscript on the toxicity of caffein, which is the first of a series of reports to be made by Dr. Salant on the pharmacology of this drug; the conclusions here reported are, therefore, in some particulars to be regarded as tentative. The data obtained are primarily of use in the execution of the food and drugs act, but are capable of much broader application.

Acknowledgment is made of the assistance rendered by Dr. John R. Mohler, Chief of the Pathological Division, Bureau of Animal Industry, and his assistants, in performing the autopsies recorded in this report. I recommend the publication of the manuscript as Bulletin No. 148 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley, *Chief.*

Hon. James Wilson,
Secretary of Agriculture.

THIS PUBLICATION may be procured from the Superintendent of Documents, Government Printing Office Washington, D. C., at 15 cents per copy

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THE TOXICITY OF CAFFEIN.

INTRODUCTION.

Comparative physiology has established the fundamental fact that some properties are common to all forms of living matter. But the same method of inquiry has also led to the recognition of marked differences in the physiological processes of various species of animals. Among the most important investigations which contributed to the knowledge of such variation of function are the studies in comparative metabolism. It is now recognized that metabolism is in some respects quite different in herbivora and in carnivora. Some forms of oxidation are much greater in the rabbit than in cats and dogs. Nuclein metabolism presents important differences in the rabbit and in man, while the mode of neutralizing acid in the body may be cited as another variation in the metabolism of these forms. Perhaps the most striking examples of differences in the metabolism of different organisms is furnished by the results of studies on the fate of certain poisons introduced into the body.

The classical experiments of Bunge and Schmiedeberg[15] [a] on the synthesis of hippuric acid are of interest in this regard. It will be recalled that in the dog the synthesis takes place in the kidney; the rabbit is able to form hippuric acid in the liver as well as in the kidney, while frogs can synthesize hippuric acid even when both of these organs have been removed or excluded from the circulation. Observations on the fate of some of the alcohols of the fatty acid series have likewise shown that these substances may be combined with glycuronic acid in some animals but not in others. According to Thierfelder and Von Mering,[84] tertiary alcohols are combined in this manner in the rabbit but not in the dog. According to Neubauer,[64] the primary and secondary alcohols are so combined in the dog as well as in the rabbit, but to a greater degree in the latter.

Pohl[73] found that amyl alcohol is largely eliminated by the lungs in the cat and in the dog. The protocols of his experiments show that 65 per cent of the alcohol given these animals was thus recovered, while he recovered only 22 per cent of this substance in the expired air of the rabbit. Examination of the urine showed the presence of glycuronic acid. Hofmeister's[37] work with tellurium in the dog is of interest in this connection. He made the important discovery that some animals possess the power of methylation as well as of demethylation. Abderhalden and Brahm's [1] experiments with pyridin show that the same is true of young dogs when on a meat diet. His experiments on rabbits with this substance were negative.

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[a] The small figures refer to the bibliography at the end of this bulletin.

The metabolism of caffein and theobromin furnish another illustration of differences in the physiological mechanism of animals. Although the substances found in the urine of man, dog, and rabbit after the administration of caffein and theobromin were the same, the quantities varied considerably. According to Krüger and Schmidt,[17] over 14 per cent of the theobromin introduced into the rabbit is eliminated as 7-methyl xanthin in the urine. The dog eliminates only about 0.67 per cent. On the other hand, the amount of tri-methyl xanthin eliminated was only 3 per cent in the dog and not quite 1 per cent in the rabbit.

It appears, therefore, from studies in comparative metabolism, whether endogenous or exogenous, that well-marked physiologic and chemical differences exist in various species of animals. That pharmacological action may likewise vary in different species of animals is shown by the following investigations. According to Guinard,[31] who made an exhaustive study of morphin, the reaction to this alkaloid varies in different forms of life, both qualitatively and quantitatively. He established its narcotic effect in the dog, rabbit, guinea pig, white mice, and rats, while for the cat, horse, ox, sheep, hog, and goat it is, on the contrary, a stimulant. Moreover, there is no evidence of cerebral effect. The stimulating effect of morphin on the nervous system in some animals was also observed by Noe [65] in experiments with this substance on the hedgehog.

Guinard [29, 30] has also shown that morphin has no narcotic effect in the marmot, although this animal is very sensitive to the drug. Two milligrams per kilo were found to be a surely fatal dose for this animal. His experiments on the comparative toxicity of morphin [30 31] show a considerable range of variation in different species. Thus the fatal dose for the dog was found to be 0.65 per kilo, while 7 mg per kilo is the fatal dose for the horse. About twice the amount is fatal for the ox and 0.2 mg per kilo kills the pig. Experiments with other drugs has shown that a considerable range of variation in resistance exists in animals of different species.

Noe's [65] studies on the comparative toxicity of chloral brought out the interesting fact that the rabbit is more resistant to it than the hedgehog and the latter more resistant than the guinea pig. Perhaps the most striking example of a difference in reaction of the same substance in widely different species is that furnished by apocodein, quinin, and yohimbin. According to Gunn [33] these substances have been found to cause vasodilation in warm-blooded animals, but they constrict the blood vessels of the frog.

Experiments with apomorphin likewise show that the reaction to this substance varies in different species of animals. The resistance of the cat to this drug is, according to Guinard,[31] ten times greater than that of the dog, and the latter is more sensitive than the rabbit to the crystalline form of apomorphin when given intravenously. According to Kobert[45] amygdalin is without effect on dogs, but it is poisonous to rabbits. Lapicque[49] found that the toxicity of curara varies in different species of frogs, the dose required to produce paralysis in *Rana esculenta* being three times greater than in *Bufo vulgaris*. Weir Mitchell[59] pointed out long ago that turtles stand enormous doses of curara. Schmiedeberg's experiments with caffein on *Rana temporaria* and *Rana esculenta* (and more recently those of Jacobi and Golowinski[42] with caffein, theobromin, and theophyllin) are also of interest in this connection. These experimenters observed well-marked differences of reaction to methylxanthins in these closely allied forms.

Experiments with quinin have shown that the action of this substance differs in some animals. It causes a fall of temperature immediately after its administration in the guinea pig, but frequently produces, at first, a rise of temperature, followed by an unimportant fall, in rabbits, dogs, and man.

The numerous investigations which have been carried out on the effect of atoxyl within recent years have contributed much to the comparative pharmacology of this substance. Although the symptoms and organic changes produced by this substance in a variety of animals present no great differences, the resistance of some has been found to vary; according to Köster[46] it is more toxic for dogs than for rabbits. A number of other substances have been found by various experimenters to vary in toxicity for different species of animals. Cantharadin, phenol, atropin, and strychnin may be mentioned as illustrations.

Pharmacological studies on lower forms of life have also revealed marked variations in the effect of some poisons. Observations made by Danilewski[18] with hydrochinone indicate that solutions of 1 to 100 or 200 are toxic to Celentrates, causing paralysis in these organisms. Echinoderms are killed within one or two hours in 1 to 1,000 or 2,000 solution, while in Vermes even weaker solutions cause tetanus and finally paralysis. The experiments of Drzewina[19] with potassium cyanid are also interesting in this connection. Teleosts placed in 100 cc of sea water containing twentieth-normal potassium cyanid showed signs of asphyxia and died in 10 to 20 minutes. Actinia placed in a solution of sea water containing five times as much potassium cyanid were active on the thirteenth day of the experiment. Similar results were obtained with other marine organisms.

From these data it is evident that the toxicity of a substance may vary considerably in different forms of life. It has been shown also by some investigations cited by Salant[78] that the action of drugs may be modified by different conditions in the environment as well as in the subject of the experiment. The recognition of the importance of these factors in determining pharmacological action has contributed much to the elucidation of the mechanism by which drugs and other substances produce physiological effects in the body. Moreover, such knowledge has often enhanced the therapeutic value of pharmaco-dynamic agents and has frequently served to avert effects of an undesirable character in man and domestic animals. The results obtained in one species of animals under a particular condition do not admit, therefore, of universal application. Furthermore, the nature of the action of a drug can only be partly learned from the manifestation of its acute effects. Equally important, therefore, especially in studies on toxicity, are the changes produced in chronic intoxication.

That the acute effects of a substance can hardly be considered a correct estimate of its toxicity is shown by the evidence obtained in experiments on tolerance and cumulative action of drugs; for the toxicity of a substance may diminish when the substance is given steadily for a long time if the body acquires tolerance for it. Arsenic, morphin, and cannabis indica may be cited as illustrations of drugs, the toxicity of which decreases with repeated doses, while digitalis and lead show a tendency to increased toxicity when similarly administered. Moreover the acute and chronic effects are sometimes qualitatively different. According to Igersheimer[41] the symptoms in acute atoxyl intoxication are nausea, vomiting, and diarrhea. These symptoms are absent in chronic intoxication, in which trophic disturbances of the skin and inflammation of the mucous membranes were the effects produced. That the acute action of atoxyl differs from the chronic effects was likewise shown by experiments on animals. The studies of von Anrep[5] on chronic atropin intoxication are of interest in this connection, as he found that after 10 to 15 injections of atropin there is no manifestation of symptoms such as is observed in acute intoxication, while the effects on the circulation are also less marked, the acceleration of the pulse being less than after the same dose in a normal subject not accustomed to its use. When the administration of atropin is continued for a longer time its usual effects on the pulse disappear altogether; there is, on the contrary a decreased frequency of the pulse. If atropin has been administered for from two to three weeks, respiration is likewise affected.

HISTORICAL REVIEW OF THE LITERATURE ON THE TOXICITY OF CAFFEIN.

Caffein was discovered in 1820 by Runge,[77] Pelletier,[69] and Robiquet[75] and was first analyzed by Dumas and Pelletier,[20] but its exact percentage composition was determined by Pfaff and Liebig,[71, 72] while to Herzog[13, 38] belongs the credit of having established that it is basic. Strecker[82] prepared caffein synthetically by heating theobromin silver and methyl iodid in a closed tube for 24 hours. Soon after its discovery in coffee Oudry[67] reported the presence of a substance in tea which he called "thein." Its identity with caffein was established 15 years later by Jobst[48] and also by Mulder.[62, 63] According to Brill,[13] Mulder (1838) was also the first to perform experiments with caffein on animals. After the administration of one-half grain to a pregnant rabbit he observed loss of appetite and kyphosis. The rabbit aborted but recovered from the effects of caffein. It has since been made the subject of numerous investigations which were carried out on a variety of animals. Observations with caffein were also made on the human subject. About four years after Mulder published his results, Lehmann[51] (1842) reported experiments on a number of people who were given caffein. The administration of from 2 to 10 grains of the alkaloid was followed by headache, palpitation of the heart, increased frequency and irregularity of the pulse, tinnitus aurium, photopsia, insomnia, and even delirium. Similar experiments reported by Frerichs[25] (1846) indicate that in doses of 25 grains it may induce severe symptoms about 15 minutes after its administration. He also observed circulatory as well as nervous symptoms and vomiting.

According to Albers[2] (1852), 4.5 grains of caffein citrate injected subcutaneously into the thigh of a rabbit was soon followed by diminished motion and tremors of the operated thigh. Other symptoms reported were spasms of the facial muscles, increased respiratory movements, and mental confusion. Of interest in this connection are the experiments of Cogswell[17] (1852) on frogs. He concluded that in point of destructive action on the tissues, caffein is far superior to morphin and may be compared to strychnin and coniin, its action on the nervous system he believed to be principally confined to the effect on the brain and spinal cord.

Lehmann[52] (1853) observed increased frequency of heart action after the administration of 4 grains, which were given with a normal diet to an adult man. When the dose was doubled the frequency of the pulse was still more increased, heart action became stronger, and tremors and confusion of thought with excitement of the imagination made their appearance. There was also an increased desire to micturate.

Stuhlmann and Falck[88] (1857) were the first to make a study of the toxicity of caffein on animals of different species. The administration of 0.5 gram of caffein subcutaneously or per rectum in rabbits induced tremors, tonic and clonic convulsions, paralysis, and increased frequency of respiration at first followed by violent dyspnœa. On autopsy he noticed congestion of the organs and in two of the three rabbits experimented upon punctiform hemorrhages of the brain with congestion of the meninges were found. In the other rabbit anemia of the brain was observed. Experiments on cats were carried out by subcutaneous, intravenous, and rectal injections. The symptoms observed after the administration of 0.5 to 0.7 gram of caffein were the same as in rabbits except that the cats developed diarrhea when caffein was given and no anatomic lesions were found on autopsy. The effect of caffein on dogs indicated that in subjects of medium weight a dose of 0.5 gram given by mouth might produce restlessness and increased frequency of respiration, while the injection of the same amount intravenously into such animals may cause death. Large, full-grown dogs, however, survived an intravenous injection of 2 grams of caffein, showing symptoms of incoordination, salivation, and frequent defecation. These investigators also made observations on caffein, using pigeons and other birds; 0.5 to 0.1 gram introduced into the stomach caused vomiting, diarrhea, tonic, but more frequently clonic, convulsions, incoordination, tremors, paresis, and paralysis.

In a few, but not in all of the birds, there was at first increased frequency of respiration followed by dyspnœa and circulatory disturbances. These amounts of caffein proved fatal in all of the experiments on birds. Inflammation of the intestinal mucosa and congestion of the meninges were the only changes found on autopsy. Stuhlmann and Falck also studied the effects of caffein on fishes and toads. Mitscherlich[60] (1858) fed 0.4 gram of caffein with bread to a rabbit and noticed lowered temperature, fatigue, convulsions, first increased then decreased frequency of respiration, and on autopsy congestion of all the viscera. He also reported observations on two frogs, one of which was given one-sixteenth of a grain of caffein in a pill with bread. It was administered to the other frog in aqueous solution, but the mode of administration was not published. The symptoms observed were in the main the same as in rabbits. In pigeons 0.125 gram introduced into the stomach caused severe vomiting, muscular incoordination, tonic rigidity of the limbs, and retraction of the head. Respiration was increased in frequency. Death followed within 3 hours and 15 minutes.

From a series of experiments on frogs which Hoppe[38] carried out (1858) by applying one-fourth of a grain of caffein to the muscles of the back, he concluded that caffein causes paralysis of the nerves, spinal cord, and brain, sensation being paralyzed before movement. The injurious action of caffein proceeds, according to Hoppe, from the spinal cord. This was based on experiments on two frogs, *Rana esculenta,* in which the right leg was amputated, the nerve being left intact, while the nerve of the other leg was ligated. At the end of 30 minutes paralysis was more marked on the right than on the left side. In another frog of the same species he resected the femoral nerve on the right side; about 1½ hours after the administration of caffein convulsions were observed. The left leg was rigid, but the right was relaxed.

Voit[85] (1860) ligated the vessels of the right lower extremity, cut the nerves of the left leg, and introduced a few drops of caffein solution into the stomach. Shortly afterwards tetanus of the right leg occurred on touching the back of the animal; the left leg was motionless. Later the entire body exhibited tetanic convulsions. From this and similar experiments Voit concluded that caffein acts first and principally on the central nervous system, and that caffein is also poisonous to nerve and muscle fibers, as they die when a solution of caffein is applied to them. The action of caffein, according to Voit, is similar in great part to that of strychnin. The effect on the blood vessels is particularly interesting, as Voit observed dilatation of the vessels, due as he thought to muscular paralysis, and also transudation and congestion of the capillaries.

Kurzak[48] (1860) made a study of the comparative toxicity of caffein in frogs and rabbits and came to the conclusion that the lethal dose for frogs is about one-seventh of that for rabbits. Caffein citrate in the form of crystals was administered in both cases by mouth. The doses given to frogs were 1 to 1.5 grains. He observed convulsions and increased respiratory activity at first; after one hour respiration diminished and voluntary muscular activity disappeared. Even on the second day convulsions were sometimes noticed. Death occurred at the end of the first or second day. Experiments on only two rabbits were reported, 0.8 gram of caffein citrate causing the death of one at the end of 13 hours. The symptoms noticed were the same practically as in frogs, but it is interesting to observe that ecchymosis of the mucous membranes of the stomach near the cardia was the only lesion found on autopsy. Several experiments made on different days on the other rabbit indicated that the toxic dose exceeded 0.5 gram, while smaller doses caused but very mild symptoms.

According to Gentilhomme[27] (1867), after caffein the reflexes are at first diminished and then disappear altogether. Death is produced by stiffness and immobility of all the muscles, particularly of the muscles of respiration, thus causing asphyxia. He furthermore held that caffein has no effect on cardiac or smooth muscle fiber, its action being specific on voluntary muscle fiber, contractions of which he observed under the microscope, thus differing completely from strychnin, which is a nerve poison.

These observations seemed to be confirmed by Pratt[74] (1868), who reported that the isolated posterior extremities and muscle fibers of the toad placed in a solution of caffein (1 grain to a wineglassful of water) for three minutes were contracted, while controls placed in distilled water were relaxed. This experiment is, of course, defective, as normal salt solution should have been used in both cases. When the muscular fibers previously immersed in caffein solution were placed under the microscope violent contractions were observed. The same author administered from 2 to 18 grains at a dose to five healthy young men. After the administration of 12 grains he noticed mental anguish, tremors of the hands and arms, and insomnia. Doses under 5 grains had no marked effect except a diminution in the frequency of the pulse and wakefulness.

About the same time Amory[4] (1868) published the results of his studies on the toxicity of caffein in cats, dogs, rabbits, and pigeons. In all cases very large doses were introduced directly into the stomach by means of a temporary gastric fistula. Ten grains given in meat to a dog caused restlessness, but no other symptoms. Doses of 30 grains and above were invariably fatal. Seventy-three grains given to a cat caused death within 20 minutes.

From observations on frogs, guinea pigs, rabbits, and on one dog, Leven[53] (1868) concluded that caffein which he gave in the form of the citrate in doses of 10 mg to frogs, from 150 to 200 mg to guinea pigs, and three to four times the latter amount to rabbits, stimulates the central nervous system and the voluntary, cardiac, and smooth muscles. He found that 0.9 gram caffein was fatal for a rabbit when injected subcutaneously, while 1 gram of the citrate was not toxic for a dog of medium size. Caffein applied directly to muscle fiber causes tetanus and destroys muscular contractility, while a nerve fiber similarly treated loses its irritability.

According to Johansen[44] (1869), caffein acts directly on the muscular fiber. After the subcutaneous injection of 0.02 gram of caffein into frogs, he observed contraction of the muscles at the site of injection, then contraction of the anterior extremities, and finally the posterior extremities become rigid and extended. Johansen observed muscular rigidity after caffein, even after curara was injected, or after ligating the vessels, or cutting the nerves which supply the muscles. He also observed that large doses of caffein diminish muscular irritability. When cardiac muscle was poisoned with caffein, microscopical examination showed that the striations disappeared. Johansen also states that reflexes disappear after caffein poisoning. He never observed tetanus in frogs, but reported tonic and clonic convulsions as a result of caffein poisoning in mammals. Somewhat different effects of caffein in frogs were observed by Buchheim and Eisenmenger[14] (1870). After the injection of 2 per cent of the citrate the frogs soon become inactive. He also observed muscular twitching of the extremities, which gradually increased, with rigidity of the muscles and opisthotonos, while respiration became slow and superficial, finally stopping altogether.

Aubert[6] (1872) studied the toxicity of caffein in man and other animals. After the ingestion of 0.36 gram, he observed dizziness, but doses of 0.12 and 0.24 gram were without any apparent effect. On the other hand, a dose of 0.5 gram of caffein was followed by increased frequency of the pulse, which soon disappeared. After one hour he noticed dizziness and trembling of the hands, which likewise passed away soon. The injection of 0.16 gram of a 2 per cent solution of caffein into the jugular vein of a rabbit weighing 1,090 grams caused tetanus and death in two and one-half minutes, and 0.12 gram injected into a rabbit weighing 980 grams caused death in one minute. Much larger doses could be borne, however, when artificial respiration was resorted to. A dog which was given 3 grams of caffein survived when artificial respiration was performed. Aubert reports, on the other hand, a similar experiment with 0.25 gram of caffein which terminated fatally.

That caffein may give rise to different effects in various species of animals was observed for the first time by Bennett.[9] He studied its action on frogs, mice, rabbits, and cats, and attempted to determine the minimum fatal dose in rabbits and cats. He also reported experiments with thein. In his first communication on the subject he states that the administration of thein to rabbits first increased and then diminished the frequency of respiration, while the pulse was decreased in frequency. Caffein, which he apparently thought was different from thein, caused increased frequency of respiration, while the pulse was markedly retarded after a preliminary acceleration. He also noticed congestion of the ears, muscular incoordination, tetanus, paralysis, diminished reflexes, and contraction of the pupils. Bennett reported the minimum fatal dose of caffein for a rabbit weighing 3.25 pounds as being 5.25–5.5 grains. The symptoms in cats after the administration of toxic doses of thein or of caffein were great excitement, paralysis alternating with convulsions, and profuse salivation. The minimum fatal dose for a cat weighing 5 pounds was, according to Bennett, 6 grains of caffein and 5.5 grains of thein. Only one experiment on a mouse is reported; the administration of 0.1 grain proved fatal. The symptoms were the same as those observed in cats and rabbits after the administration of caffein. The experiments on frogs indicate that the symptoms were about the same as those previously described in the case of warm-blooded animals except that the reflexes are almost completely lost after the subcutaneous injection of doses of one-sixteenth to one-twelfth of a grain. The latter dose was fatal for frogs. It would be of interest to know the comparative toxicity of caffein to frogs and mammals, but unfortunately the weights were not reported.

Schmiedeberg[79] (1874) noticed that the administration of 20 mg of caffein to frogs weighing about 45 grams was followed, in *Rana esculenta,* in about 25 minutes, by increased reflexes, 7 minutes later by tetanus. Several attacks occurred, but tonic spasms were never observed. On the contrary, when the same amount of caffein was given to *Rana temporaria* weighing 45 grams he noticed a marked diminution of the reflexes and tonic rigidity of the muscles after 23 minutes; the reflexes were greatly increased, however, about 24 hours later. The frogs were under observation for three days, and although symptoms were still present at the end of this time in the subjects of both species tetanus was never observed in *Rana temporaria.*

Peretti's[70] (1875) studies on the effects of caffein were confined chiefly to observations on dogs. He also made observations on a few rabbits and reported an experiment on one cat to which he administered, by subcutaneous injection, 0.18 gram of caffein per kilo and noticed increased frequency in lachrymation and crying. The cat was found dead the next day. The subcutaneous injection of a rabbit in which artificial respiration was instituted with 0.36 gram of caffein per kilo proved fatal soon after the injection without any manifestation of symptoms. Small doses of caffein, 0.1 gram, given to a rabbit weighing 3,670 grams, failed to produce any visible effects. Doses under 0.1 gram per kilo likewise failed to induce any symptoms in dogs. When 0.1 gram of caffein per kilo was given by mouth or subcutaneously it was followed by restlessness, salivation, rigidity of hind legs, and vomiting. In both instances the dogs recovered. The symptoms were more severe when the dose was increased to 0.185 gram per kilo, but even in this case the dog recovered. A dose of 0.2 gram per kilo, however, proved fatal.

Henneguy[36] (1875) experimented on three frogs to which he gave 0.01 gram of caffein citrate subcutaneously. He observed mild stimulation of the nervous system and of the muscles, as well as increased cardiac activity. Later, voluntary movement and respiration disappeared and sensations diminished, but convulsions of the extremities appeared. Cardiac activity was then diminished, the heart being finally arrested in systole. Since the motor nerves retained their irritability even after the reflexes disappeared, he concluded that the loss of motion was due to the action of caffein on the nerve centers.

Binz[11] (1878) reported experiments on dogs and also made some observations on man with caffein. The subcutaneous injections of 0.2 gram caffein may prove fatal to dogs, although some survive such a dose. The toxic dose in man varies from 0.5 to 1.5 grams. Disturbance of the circulation, such as palpitation of the heart and fullness of pulse, restlessness, and diarrhea were the symptoms he observed.

Extensive investigations on the action of caffein were carried out by Leblond[50] (1883), who studied its effect on the circulation in man and lower animals, and its toxicity in the lower animals alone. Five to twenty centigrams of caffein and 0.06 to 0.25 gram of salicylate of soda were dissolved and injected into the muscles of the thigh of young guinea pigs weighing a little over 300 grams. In the three experiments reported the death of the animals occurred after 23 minutes, 40 minutes, and 1 hour and 20 minutes. Symptoms appeared in from 10 to 15 minutes after the injection of caffein. Incoordination of movements, convulsions, both tonic and clonic, opisthotonos, tremors, increased frequency of respiration, ataxia, paralysis were the symptoms observed. It is worthy of note that the appearance of paresis preceded the convulsions. Diminished sensation was reported in one pig, but no sensory disturbances nor reflexes had been observed in the other. Two rabbits, one of which received 0.5 and the other about 0.3 gram of caffein per kilo with equal parts of salicylate of soda, were injected subcutaneously into the thigh. Diminished sensation, paresis of the posterior extremities, hyperexcitability, convulsions, opisthotonos, dilation of the veins of the ear were observed. Death followed in 1 hour and 23 minutes in one rabbit and in 3 hours and 7 minutes in the other.

Filehne[22] (1886) experimented with caffein on *Rana esculenta* and *Rana temporaria*. The subcutaneous injection of 7 mg of caffein into *Rana esculenta* caused tetanus, while 50 mg given by mouth caused tonic spasms. He further stated that the difference between *Rana esculenta* and *Rana temporaria* as regards the reaction to caffein was one of degree only.

Amat[3] (1889) reported experiments on three guinea pigs, in which 0.4 to 0.5 gram per kilo injected subcutaneously proved fatal within 38 and 44 minutes. One guinea pig which received 0.1 gram of caffein per kilo survived. The symptoms observed in the two fatal cases were general muscular rigidity and convulsions.

Parisot[68] (1890) made a study of the toxicity of caffein on different species of animals. Unlike most of his predecessors, however, he reported, at least in some cases, the weight of the animals on which he worked. After the subcutaneous and intramuscular injections of from 5 to 20 mg of caffein into *Rana temporaria* weighing from 14 to 16 grams, he noticed increased irritability at first; later, a loss of reflexes, inability to use the muscles, complete muscular rigidity resembling rigor mortis, and also cessation of heart action. The effect of caffein produced in the green frog was analogous to that observed in strychnin poisoning. Parisot found, however, that muscular rigidity developed, although very gradually, also in the green frog, but it set in much later than in frogs of the other species and without superseding the clonic convulsions. According to Parisot, the muscular rigidity after caffein persists after the destruction of the brain and spinal cord, thus showing that it is not of nervous origin. He further emphasized the difference in the behavior of these two species of frogs toward caffein by stating that he never observed tetanic convulsions in the red frog. His experiments also indicate that the green frog is more resistant to caffein than *Rana temporaria*, as the same doses which are fatal for the latter were only toxic for *Rana esculenta*. The number of experiments, however, is too few to justify a positive conclusion on this point. Parisot also made some experiments on turtles. The results he obtained show that caffein is at least as toxic for these animals as for the frogs he experimented upon, 0.33 gram per kilo (carapace not included in weight) having proved fatal within 24 hours. Two experiments on one pigeon were also reported by the same observer; two doses of 0.06 gram per kilo given at an interval of four hours caused mental depression and muscular rigidity, but the pigeon survived.

Experiments with caffein on the human subject made by Parisot showed that man is far more susceptible to this substance than the other animals he investigated. After the ingestion of 0.3 gram of caffein symptoms of intoxication pointing to cerebral disturbance appeared, which became more marked when the size of the doses was increased.

It will be noticed that the nature of the action of caffein, whether it is a nerve or a muscle poison, formed the subject of several investigations. Binz [11] (1890) brought forward additional evidence in support of the view that caffein acts primarily on the ganglion cells, and not on the muscle directly. This he has shown by injecting 0.5 gram into each of two rabbits after cutting the sciatic nerve on one side; in one case he also resected the obdurator and crural nerves on the same side. Clonic spasms developed in both subjects soon after caffein was given, but in each rabbit the side operated upon remained paralyzed. Baldi [8] (1891) studied the action of caffein on *Rana esculenta*. After injecting from 4 to 20 mg tetanus, such as observed in strychnin poisoning, was noticed. Fröhner [26] (1892) made observations on the comparative toxicity of caffein in domesticated animals. After the administration of 5 grams of caffein sodium salicylate by mouth to a dog weighing 10 kilos, he noticed salivation, restlessness, vomiting, and convulsions as in strychnin poisoning. Death occurred three hours after the drug was given. On autopsy he noticed mild inflammation of the mucous membranes of the stomach and intestines and edema of the lungs; the heart was in diastole. A dose of 2 grams of caffein sodium salicylate given to the same animal subcutaneously two days previously provoked only very slight symptoms. The subcutaneous injection of 10 grams of the same preparation into a pig weighing 30 kilos caused death in two and a half hours, with the production of symptoms of disturbance of the nervous system and of gastrointestinal irritation. The same dose per kilo of body weight given to a goat likewise caused death in two and a half hours after its administration. Examination on autopsy revealed inflammation of the gastrointestinal tract. Similar lesions were found in a horse killed by 100 grams of caffein, in which he also noticed hemorrhage of the mucosa in the fundus of the stomach.

Gourewitch[28] (1907) conducted experiments with caffein on rabbits, pigeons, and white rats. It appears from his protocol that single doses of about 0.2 to 0.25 gram caffein per kilo given subcutaneously proved to be fatal. He states, however, that the resistance to caffein was markedly diminished, when its administration was repeated daily, for much smaller amounts sufficed to cause death in these animals. A dose of 120 mg of caffein per kilo proved fatal after the third injection. When the dose was increased to 170 mg per kilo, the animal succumbed to the effects of caffein after the second injection. His experiments on the other animals do not indicate the degree of resistance to caffein, since the weights for some were not given while for the others no attempt was made to determine the minimum toxic or fatal dose.

Maurel[55] (1907) studied the influence of different methods of administration on the toxicity of caffein on frogs and rabbits. He determined the minimum toxic and lethal doses of caffein hydrobromid which he employed in 1 to 2 per cent solutions. He concluded from his experiments that the toxicity of caffein when given by mouth is twice as great for the frog as for the rabbit.

More recently Hale[33] carried out a number of experiments on guinea pigs in which he determined the toxicity of caffein given in the form of the citrate and made into a pill with mucilage of acacia and arrowroot starch. After the pill was dried it was fed to the animal, due precaution being taken that none of it was lost during feeding. From experiments on guinea pigs which received doses of 0.3 to 0.6 gram caffein citrate, the following data have been reported: Three decigrams per kilo given to one pig was not fatal. Of three pigs which received 0.4 per kilo, one died and two survived. Exactly the same results were obtained in three others which received 0.5 per kilo. Two guinea pigs, which received 0.55 and 0.6 per kilo each, died after 15 and 7 hours, respectively, while another animal survived a dose of 0.45 per kilo.

This review of the literature on the toxicity of caffein, although bearing evidence of considerable investigation and extending over three-quarters of a century, is largely qualitative in character. It appears from the experiments that the main object of the investigations was to ascertain the nature of the action of caffein, whether it is a muscle or a nerve poison. The comparative toxicity in different species of animals by the accurate determination of the toxic and fatal doses received but little attention. To fill the gap in our knowledge of the toxic effects of caffein, the present investigation was undertaken. This, it will be seen, proved to be a most laborious task, because in the large number of experiments careful observations showed that individuals of the same species varied considerably in their reaction to the drug. Numerous other factors, as will be shown, were also found to play an important part in the determination of the toxicity of caffein.

ACUTE CAFFEIN INTOXICATION.

The object of these experiments was to determine the resistance to caffein in various species of animals and by various methods of administration. Caffein was therefore given by mouth and injected subcutaneously into the peritoneal cavity, into the muscles, and intravenously. As far as could be judged by appearance, healthy animals were selected for the subjects of the experiments, but as it is impossible to diagnose with any degree of accuracy the condition of the animal while it is alive, post mortem examinations were resorted to in many cases in which the issue of the experiment was fatal. Since the age of the animal may modify toxicity full grown, as well as young, animals were employed for these experiments; diet, race, and season also play an important part in determining the toxicity of a drug and these factors were also taken into account in the present investigation.

EXPERIMENTS ON RABBITS.

Animals of different varieties were used and were given caffein by all of the methods indicated in the preceding paragraph. Some of the rabbits employed in these experiments received oats, others received a diet exclusively of carrots for several days or weeks previous to the administration of caffein. The experiments were conducted at all seasons of the year.

SUBCUTANEOUS INJECTION.

From a study of the literature on the toxicity of caffein it seemed that about 150 mg per kilo is probably the lethal dose for the rabbit when the drug is injected subcutaneously. Preliminary observations were therefore carried out with such a dose, but it was found, on the contrary, that this amount per kilo was hardly sufficient to induce symptoms in the great majority of cases.

SERIES A.

[Doses of 147 to 167 mg of caffein per kilo were employed in these experiments.]

Rabbit 332. Belgian hare, female. Weight, 1,070 grams. Diet, oats.
March 25: 8.5 cc 2 per cent caffein (158 mg per kilo) injected subcutaneously at 2.15 p. m.; 4 p. m., reflexes increased; 5.45 p. m., increases of reflexes still more marked.
March 26: Rabbit looked normal; no symptoms observed.

Rabbit 331. Belgian hare, female. Weight, 1,170 grams. Diet, oats.
March 25: 2.15 p. m., 9 cc 2 per cent caffein (153 mg per kilo) injected subcutaneously; 4 p. m., reflexes increased; 5.45 p. m., condition the same.
March 26: Rabbit looks normal; no symptoms observed.

Rabbit 328. Belgian hare, female. Weight, 1,200 grams. Diet, oats.
March 25: 9 cc 2 per cent caffein injected subcutaneously (150 mg per kilo); 4 p. m., reflexes increased; 5.45 p. m., reflexes increased but not markedly.
March 26: No symptoms; rabbit looks normal.

Rabbit 322. White female. Weight, 1,065 grams. Diet, oats.
March 17: 8 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously at 11.55 a. m.; 12.55 p. m., reflexes increased, but no tetanus nor any other symptoms.
March 18: Rabbit running around in cage; condition apparently normal.
March 25: Condition of rabbit good.

Rabbit 217. White. Weight, 1,355 grams. Diet, oats.
October 29: 10 cc 2 per cent caffein (147 mg per kilo) injected subcutaneously at 1.51 p. m. 5.15 p. m., rabbit alive; survived.

Rabbit 219. Maltese. Weight, 1,820 grams. Diet, oats.
October 29: 14 cc 2 per cent caffein injected subcutaneously at 1.40 p. m. (153 mg per kilo); 5.15, rabbit alive; survived.

Rabbit 194. White female. Weight, 1,490 grams. Diet, oats.
October 14: 13 cc 2 per cent caffein (174 mg per kilo) injected subcutaneously; increased reflexes and tremors were observed.
October 15: Condition of rabbit good; no symptoms.

Rabbit 191. Brown male. Weight, 1,915 grams. Diet, oats.
October 14: 16 cc 2 per cent caffein (167 mg per kilo) injected subcutaneously; reflexes increased and tremors present.
October 15: Condition of rabbit good.

A study of this series shows that about 150 mg of caffein per kilo caused increased reflexes within one to two hours after injection. When the dose was increased, as in rabbits 194 and 191, the symptoms were more pronounced; 150 mg per kilo may be regarded as the minimum dose which produces symptoms of nervous irritability when caffein is injected subcutaneously. Experiments with larger doses were therefore carried out in order to determine the minimum fatal dose.

SERIES B.

Approximately 0.2 gram of caffein per kilo was employed in these experiments. Diet and race as possible factors which may influence the toxicity of caffein were made the subject of study in these experiments which were divided into two groups as shown in the table, page 25.

Rabbit 95. Gray and white male. Weight, 1,478 grams. Diet, oats.
February 27: 11.30 a. m., 15 cc 2 per cent caffein (210 mg per kilo) injected subcutaneously; 2.20 p. m., no symptoms, tremors observed when handled, but not marked, reflexes slightly increased, no muscular rigidity nor any other symptoms; 2.45 p. m., rabbit suddenly became very restless, jumped off the table, and had convulsions; 3.45 p. m., rabbit died, rigor mortis set in almost immediately after death.

Rabbit 96. Gray and white male. Weight, 1,585 grams. Diet, oats.
February 27: 16 cc 2 per cent caffein (200 mg per kilo) injected subcutaneously at 3.40 p. m.; increased reflexes observed about one hour after caffein was injected, but no other symptoms.
February 28: Rabbit found dead.

Rabbit 112. Black female. Weight, 875 grams. Diet, oats.
March 18: 9 cc 2 per cent caffein (205 mg per kilo) injected subcutaneously at 3 p. m.; 3.30 p. m., rabbit became restless, reflexes were increased, tremors were

observed, but no other symptoms; 4.15 p. m., rabbit had tremors, was handled but this failed to induce tetanus, 10 minutes later tetanus of short duration with recovery occurred.

March 19: 9 a. m., found dead.

Rabbit 119. Yellow white female. Weight, 1,060 grams. Diet, oats.

April 17: 10 cc 2 per cent caffein (188 mg per kilo) injected subcutaneously at 2.10 p. m.

April 18: Rabbit found dead.

Rabbit 195. White female. Weight, 1,300 grams. Diet, carrots, since October 7.

October 14: 13 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously at 11.15 a. m.; 2.25 p. m., rabbit had convulsions and died. *Note:* Ulceration of rectum was noticed.

Rabbit 208. Gray. Weight, 1,068 grams. Diet, carrots, October 7–15, inclusive.

October 15: 10 cc 2 per cent caffein injected subcutaneously at 11 a. m.; 1 p. m., increased reflexes and tremors observed; 3.45 p. m., tremors were marked when rabbit was handled.

October 16: Rabbit found dead. *Note:* Looked poorly nourished.

Rabbit 247. Belgian hare, female. Weight, 1,295 grams. Diet, oats last 10 days before experiment.

November 10: 11 a. m., urine obtained from the bladder was acid to litmus and did not contain sugar or albumen, 13 cc 2 per cent caffein was injected subcutaneously; 1.30 p. m., 15 cc urine obtained was markedly alkaline to litmus and reduced Fehling's solution; 2.30 p. m., reduction of urine considerable, marked tremors observed but no tetanus.

November 11: 10.30 a. m., 95 cc urine collected gave moderate reduction of Fehling's solution, no symptoms, condition of rabbit seemed to be good.

Rabbit 248. Belgian hare, female. Weight, 1,305 grams. Diet, oats the last 10 days before the experiment.

November 10: 11 a. m., urine markedly acid to litmus, no albumen, no sugar, 13 cc 2 per cent caffein injected subcutaneously; 1.30 p. m., urine was slightly alkaline to litmus, no reduction of Fehling's solution; 2 p. m., reflexes increased; 2.30 p. m., 2 cc urine obtained from bladder, sugar abundant; 4.45 p. m., reflexes increased as before, but no tetanus.

November 11: 10.30 a. m., urine collected showed slight reduction of Fehling's solution; otherwise condition of rabbit was good; rabbit did not show any effects of caffein.

Rabbit 337. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously in the back (0.211 per kilo); 4.30 p. m., reflexes much exaggerated.

April 7: 8.15 a. m.; condition good, no symptoms.

Rabbit 336. Belgian hare. Weight, 1,040 grams. Diet, carrots, March 31 to April 6, inclusive.

April 6: 3 p. m., 11 cc 2 per cent caffein injected subcutaneously into tissues of the back.

April 7: 8.15 a. m., no symptoms, condition good.

Although symptoms appeared in rabbits of Group I (see table, page 25) about the same time after the administration of caffein as in the rabbits of the preceding series all of them terminated fatally 2¼ hours to 24 hours after its administration. Two of these rabbits (Nos. 195 and 208) were fed carrots for several days before the injection of caffein, the others were fed oats. Since symptoms and death appeared in these two rabbits about the same time as in the rest of this group it may be concluded that caffein is not less toxic when carrots are fed than when oats form the exclusive diet. But since rabbit No. 208 was poorly nourished and ulceration of the rectum was observed in No. 195 it is quite possible that caffein might be less toxic in normal rabbits on this diet. This was tested in rabbits Nos. 336 and 337, both of which seemed to be free from abnormality and were well nourished. Since these rabbits survived and manifested mild symptoms only of intoxication it would seem that a carrot diet decreases the toxicity of caffein.

It was suggested, however, that another factor might be the cause of the greater resistance to caffein in these two rabbits, namely, race. This was tested in rabbits 247 and 248, both Belgian hares. Since the toxicity of caffein in these two rabbits was the same as in Nos. 336 and 337, diet as a factor in acute caffein intoxication may be disregarded. The greater resistance to caffein of these four rabbits is in all probability due, therefore, to a difference of race. This suggestion gained additional support from the experiments of the next series.

SERIES C.

The object of these experiments was to determine the minimum fatal dose for the gray rabbit and to obtain additional evidence as to the toxicity of caffein in the several varieties of rabbits. Eight experiments were performed, in which from 236 to 252 mg per kilo were given. The white rabbits, three in number, received 250, 242, and 238 mg per kilo. All the others (which were Belgian hares) received from 236 to 252 mg per kilo. Two of the white rabbits were fed carrots for one week preceding the injection of caffein. The other was fed oats. Three of the Belgian hares were on a diet of oats, two were fed carrots the week before the experiment with caffein.

Rabbit 122. White, female. Weight, 2,060 grams. Diet, oats.

April 14: 25 cc of 2 per cent caffein (250 mg per kilo) in aqueous solution injected subcutaneously in the back at 1.35 p. m.; 4.30 p. m., tremors, reflexes increased, condition otherwise good.

April 16: 9 a. m., found dead in cage. *Autopsy:* Liver deeply congested; kidneys congested in cortex and medulla; stomach showed small hemorrhagic areas, perforating ulcers in pyloric portion; small intestine petechiated on mucosa; lungs and spleen normal.

Rabbit 234. White, female. Weight, 1,650 grams. Diet, November 2–9, carrots.

November 9: 10.45 a. m., 20 cc 2 per cent caffein (242 mg per kilo) administered subcutaneously.

November 10: 9 p. m., found dead.

Rabbit 335. Gray hare, female. Weight, 1,170 grams. Diet, March 31 to April 7, carrots.

April 7: 9.30 a. m., 14 cc 2 per cent caffein solution (240 mg per kilo) injected subcutaneously in the back; 10.30, reflexes much increased, rabbit is extremely sensitive.

April 8: 9 a. m., found dead. *Autopsy:* Liver was congested and contained several coccidiosis nodules; stomach distended with rather dry food mass; mucosa exhibited mild catarrhal inflammation; mucosa of intestines also slightly inflamed.

Rabbit 249. Belgian hare, female. Weight, 1,185 grams. Diet, oats.

November 11: Urine, 5 cc, from bladder acid to litmus, no sugar, no albumin; 11.50 a. m., 14 cc 2 per cent caffein (236 mg per kilo) administered subcutaneously; 3.45 p. m., reflexes increased, hyperæsthesia marked, but no tetanus, even when handled; 30 cc urine collected at 4 p. m., reduction of Fehling's solution considerable.

November 12: 10 a. m., 8 cc urine collected, reduction heavy, only a few cubic centimeters obtained from bladder, did not contain any sugar, general condition of rabbit good, no symptom of caffein intoxication.

Rabbit 321. Yellow, female. Weight, 1,135 grams. Diet, oats.

March 16, 1910: 11.50 a. m., 14 cc 2 per cent caffein (246 mg per kilo) injected subcutaneously in the back; 2 p. m., reflexes increased, is very sensitive, started to run when put on floor, no handling except what was required for removal and return to cage, feces soft.

March 17: 9.30 a. m., condition good, rabbit put on floor, gait normal, but does not care to walk.

March 18: 9 a. m., walks around when put on floor, appetite good, condition seems to be normal.

March 25: 11 a. m., rabbit still alive, condition good.

Rabbit 250. Belgian hare, female. Weight, 1,435 grams. Diet, oats at least two days before the experiment.

November 11: 11 a. m., urine obtained from bladder acid to litmus, no albumin, no sugar; 11.10 a. m., 18 cc, 2 per cent caffein (252 mg per kilo); 3.45 p. m., reflexes and hyperæsthesia, no tetanus; 4 p. m., 60 cc urine, marked reduction of Fehling's solution.

November 12: 10 a. m., condition of rabbit good, no symptoms of caffein intoxication, 80 cc urine collected, sugar considerable, only a few cubic centimeters of urine obtained from bladder, no reduction of Fehling's solution.

Rabbit 334. Belgian hare, female. Weight, 1,270 grams. Diet, carrots, March 31 to April 7.

April 7: 9.30 a. m., 15 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously in the back; 10.30 a. m., reflexes much increased, rabbit extremely sensitive.

April 8: 9 a. m., condition good, no symptoms.

Rabbit 233. White, male. Weight, 1,675 grams. Diet, carrots, November 2 to 9.

November 9: 10.50 a. m., 20 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously, no symptoms observed until 5 p. m., when increased reflexes and hyperæsthesia were noticed, but no tetanus.

November 10: 9 a. m., paralysis of posterior extremities; died at 1 p. m.

Analysis of the results obtained in the experiments of this series and inspection of Table I, page 25, show that all four of the rabbits which survived doses of 236 to 252 mg of caffein per kilo were Belgian hares. Of the four which died one only was a Belgian hare. The other three were white rabbits. Two of these were fed oats; the other two received carrots during seven days preceding the administration of caffein. This diet does not seem to be a factor, therefore, in the toxicity of caffein. Moreover, it may be observed that rabbit No. 122, which was fed oats, died after receiving 250 mg per kilo, while rabbit No. 250 received the same diet and survived the same dose of caffein per kilo.

Experiments 234 and 334 offer another illustration that the toxicity of caffein is not dependent upon diet, since both rabbits were fed carrots, but the same dose of caffein caused only symptoms in one while it proved fatal to the other. It is evident, therefore, that the difference in resistance to caffein shown in these experiments is in all probability due to race, the Belgian hare being more resistant to caffein than rabbits of other varieties. Rabbit No. 335 seems to be an exception, but the post-mortem examination showed the presence of coccidiosis of the liver. As will be shown later, wherever this condition prevailed even smaller doses of caffein proved fatal.

SERIES D.

To obtain additional evidence regarding the resistance of the various races of rabbits to caffein and to ascertain the smallest dose which is surely fatal to the gray rabbit or Belgian hare was the object of this series of experiments. The diet in all cases consisted of oats, which was given ad libitum excepting to rabbit No. 235, which received carrots for one week previous to the injection of caffein. The doses administered ranged from 267 to 300 mg per kilo and were administered to different varieties of adult rabbits.

Rabbit 253. Brown and black, male. Weight, 1,600 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., urine from bladder acid, no albumen, no sugar; 11.35 a. m., 22 cc 2 per cent caffein (275 mg per kilo) injected subcutaneously; 11.45 a. m., rabbit jumped off the table, had convulsions, retraction of head and opisthotonos, general tremors, anterior extremities stretched out, posterior extremities almost normal, frequent twitchings; died at 12.15 p. m.

Rabbit 252. Black, female. Weight, 1,335 grams. Diet, oats, November 9 to 12.

November 12: 11.30 a. m., 18 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously. Urine obtained from bladder before injection, acid, no albumen, no sugar, color normal, tremors and great excitement noticed about 12 noon; 4.30 p. m., when handled, showed unusual restlessness and excitement followed by convulsions with opisthotonos; occasional twitching, condition bad. Died 4.35 p. m.

Rabbit 327. White, female. Weight, 820 grams. Diet, oats, March 8 to 16.

March 16: 11.45 a. m., 12 cc 2 per cent caffein (292 mg per kilo) injected subcutaneously in the back; 2 p. m., found dead, but was still warm. *Autopsy:* Hemorrhagic area at point of injection into spinal muscles; subcutaneous abdominal region exhibited a large area of cheesy purulent material; liver and spleen were engorged; bladder filled; intestines normal.

Rabbit 340. White and brown male. Weight, 1,465 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc of 2 per cent caffein (273 mg per kilo) injected subcutaneously in back.

March 31: 9 a. m., found dead.

Rabbit 341. White and brown. Weight, 1,450 grams. Diet, oats.

March 30: 3.20 p. m., 20 cc 2 per cent caffein (270 mg per kilo) injected subcutaneously in back; 4.40 p. m., found in dying condition, had convulsions; 4.45 p. m., dead.

Rabbit 326. White, male. Weight, 1,645 grams. Diet, oats, March 8 to 16.

March 16, 1910: 12 noon, 20 cc 2 per cent caffein (243 mg per kilo) injected subcutaneously in the back; 2 p. m., tremors marked, hypersensitive, started to run when put on floor; rabbit was not handled any more than was required for his removal from and return to cage.

March 17: 9.30 a. m., tremors still present and marked, otherwise general condition good; no other symptoms.

March 18: 9.30 a. m., no appetite, tremors still present, general condition poor; died about 2 p. m.

Rabbit 235. Belgian hare, male. Weight, 1,870 grams. Diet, carrots, November 2 to 9.

November 10: 11.05 a. m., 25 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously; reflexes increased and tremors, but no tetanus observed; found dead next morning.

Rabbit 316. Belgian hare, female. Weight, 860 grams. Diet, oats, March 8 to 16.

March 16, 1910: 11.40 a. m., 12 cc 2 per cent caffein (267 mg per kilo) injected subcutaneously in the back; 2.15 p. m., reflexes somewhat increased, but not markedly so; walked when put on floor; gait clumsy and slow; tremors of head observed; 2.35 p. m., rabbit lying in his cage, posterior extremities extended and rigid, anterior extremities flexed, head retracted; is still breathing; occasional spasms observed. Rabbit died at 3 p. m. *Autopsy:* No lesion at point of injection in dorsal spinal muscles; liver and spleen engorged; intestines injected; other organs apparently normal.

Rabbit 395. Belgian hare, male. Weight, 1,410 grams.

August 18: 1 p. m., 20 cc 2 per cent caffein (283 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes markedly increased; 5 p. m., reflexes about the same, but no tetanus.

August 19, 9.15 a. m.: Reflexes increased markedly.

August 21, weight, 1,215 grams. Given 275 mg per kilo of caffein; no symptoms observed.

August 23, found dead. *Autopsy:* Liver greatly engorged; stomach fairly well distended and mucous membrane in a slightly inflammatory condition; contents of small intestine liquid in nature, but walls of same appeared normal; other organs normal in appearance.

Rabbit 396. Belgian hare, female. Weight, 1,475 grams. Diet, oats.

August 18: 1 p. m., 20 cc 2 per cent caffein (272 mg per kilo) injected subcutaneously in the back; 4 p. m., reflexes increased markedly; 5 p. m., reflexes increased markedly but no tetanus.

August 15: 10.30 a. m., reflexes still increased very markedly; rabbit jumps when touched.

August 21: Weight, 1,245 grams. Injected subcutaneously 275 mg of caffein per kilo; reflexes increased, posterior extremities stiff over hour later.

August 22: 9 a. m., found dead. *Autopsy:* Thoracic organs normal in appearance; stomach distended and mucous membrane affected with a catarrhal inflammation; contents of stomach were covered with a shiny mucus; contents of small intestine liquid in nature and bile stained; liver showed a coccidial infestation; kidneys and spleen normal in appearance.

Rabbit 397. Belgian hare, male. Weight, 1,375 grams. Diet, oats.

August 19: 10.30 a. m., 20 cc 2 per cent caffein (290 mg per kilo) injected subcutaneously in the back.

August 22: 9 a. m., found dead. *Autopsy:* Stomach distended with ingesta; mucous membrane exhibited a catarrhal inflammation with excessive secretions; major portion of intestines showed a condition similar to that of stomach, contents consisting mainly of a shiny mucus; liver enlarged; other organs apparently normal.

Rabbit 398, Belgian hare, female. Weight, 1,570 grams. Diet, oats.

August 19: 10.30 a. m., 23 cc 2 per cent caffein (293 mg per kilo) injected subcutaneously in the back; 4 p. m., found dead. *Autopsy:* Thoracic organs seemingly normal; mucous membrane of stomach exhibited a catarrhal inflammation generally; large intestines somewhat impacted but walls appeared normal; other organs normal.

Rabbit 399, Belgian hare, male. Weight, 1,725 grams. Diet, oats.

August 19: 10.30 a. m., 26 cc 2 per cent caffein (300 mg per kilo) injected subcutaneously in the back; found dead at 4.30 p. m. *Autopsy:* Lungs slightly congested; liver engorged and friable; gall cyst well filled; stomach exhibited catarrhal gastritis; injection of mesenteries and intestines; kidney showed marked cortical congestion.

The results of the experiments of this series likewise indicate that the Belgian hare is more resistant to caffein than the rabbits of other varieties. Thus, of the four gray rabbits (Nos. 235, 316, 395, and 396), which received 267 to 283 mg of caffein per kilo, two died and two lived,[a] one of which, 396, showed the presence of coccidiosis of the liver. On the other hand it will be observed that the black and white rabbits which received from 270 to 275 mg of caffein per kilo all died from the effects of the drug; one within 1 hour and 25 minutes and another within 50 hours after the administration of the caffein, while No. 340 died in the night. Furthermore it will be noted that of the last three rabbits of this series, which were Belgian hares and received 290, 293, and 300 mg of caffein, two died six hours after the injection, while the other, No. 397, lived three days. The minimum fatal dose of caffein for Belgian hares is, therefore, about 290 to 300 mg per kilo when injected subcutaneously, which is about 50 per cent greater than for rabbits of other varieties.

SERIES E.

It was shown in series A that 0.15 caffein per kilo caused symptoms of intoxication. Before concluding, however, that this is the smallest dose which causes symptoms of poisoning, a number of experiments were performed with smaller doses. It was found that in the great majority of cases 0.1 caffein per kilo may cause diuresis, but no nervous or muscular symptoms. In some rabbits, however, even such a dose proved fatal. Post-mortem examinations in these cases showed the presence of coccidiosis of the liver, and it will be recalled that similar observations were made before. It is quite possible, therefore, that coccidiosis of the liver is an important factor in decreasing the resistance to caffein. Experiment 551 (p. 25) shows that other conditions may likewise increase the toxicity of caffein.

Rabbit 325. White, female. Weight, 1,065 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent (112 mg per kilo) caffein injected subcutaneously in the back. About 5 cc of urine squeezed out from bladder before injecting caffein.

March 17: 1 p. m., hind legs crossed and stretched out, front legs also extended; rabbit lying stretched out on her belly.

March 17: 5.40 p. m., rabbit still alive, condition somewhat improved.

March 18: 9 a. m., found dead, stiff and cold. *Autopsy:* Hemorrhagic area at point of inoculation; subcutaneous region of both thighs presented a hemorrhagic infiltration of the tissues; liver contained lesions of coccidiosis; other organs apparently normal.

Rabbit 330. Belgian hare, female. Weight, 935 grams; poorly nourished.

March 18: 3.35 p. m., 5 cc 2 per cent caffein (107 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Weight, 825 grams.

Rabbit 329. Belgian hare, male. Weight, 775 grams; poorly nourished. Received March 18.

March 18: 3.30 p. m., 4 cc 2 per cent caffein (103 mg per kilo) injected into subcutaneous tissues in the back; 5.30 p. m., no symptoms.

March 19: 9 a. m., no symptoms.

March 25: Rabbit alive in good condition; weight, 825 grams.

Rabbit 320. Black, male. Weight, 1,040 grams. Diet, oats.

March 17: 11 a. m., 6 cc 2 per cent caffein (115 mg per kilo) injected subcutaneously in the back; only a few drops of urine obtained from bladder before injecting caffein; 1 p. m., rabbit very restless; ran away when placed on floor; cried when touched with a piece of paper; no tremors observed, but rabbit became exhausted and was unable to walk; legs extended out; after running for about a minute dyspnœa was very marked, but rabbit soon raised himself on his legs; 5.40 p. m., rabbit up on his legs.

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[a] Survived first dose.

March 18: 9 a. m., found dead, but still warm. *Autopsy:* Lungs studded with small grayish white nodules, adhesions to costal pleura; probably lesions of coccidiosis; liver studded with coccidiosis nodules. Hemorrhages at point of inoculation.

Rabbit, 551. Gray, female. Weight, January 26, 1,650 grams. Diet, oats; fed 20 cc of 25 per cent alcohol daily from January 26–31.

January 31: Weight, 1,450 grams; 10.20 a. m., temperature 101.6°; 10.45, a. m., temperature 101.6°; received 7 cc 2 per cent caffein subcutaneously into back; 11.15 a. m., convulsions of short duration; raised himself on posterior legs, anterior legs wide apart; 4.10 p. m., looked normal, not hypersensitive; 4.30 p. m., condition seemed to be good.

February 1: 9 a. m., found dead, was alive at 5.30 p. m. of previous day. *Autopsy:* Lesions found involved thoracic cavity mainly; lungs were hepatized and a fibro plastic exudate caused them to adhere to costal pleura; liver engorged and appeared fatty; no marked lesions affecting digestive tract, a slight catarrh of stomach being the only noticeable feature; kidneys and spleen normal.

TABLE 1.—*Subcutaneous injections of caffein—rabbits.*

SERIES A.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
332	1,070	158	1 hour 45 minutes	Survived	Oats	Gray.
331	1,170	153	do	do	do	Do.
328	1,200	150	do	do	do	Do.
322	1,065	150	1 hour	do	do	White.
217	1,355	147		do	do	Do.
219	1,820	153		do	do	Maltese.
194	1,490	174		do	do	White.
191	1,915	167		do	do	Light brown.

SERIES B, GROUP I.

95	1,478	210	2 hours 50 minutes	3 hours 10 minutes	Oats	White.
96	1,585	200	1 hour	About 18 hours	do	Gray white.
112	875	205	30 minutes	do	do	Black.
119	1,060	188		do	do	Yellow white.
195	1,300	200		8 hours 10 minutes	Carrots	White.
208	1,068	188	2 hours	About 24 hours	do	Gray.

SERIES B, GROUP II.

247	1,295	200	2.5 hours	Survived	Oats	Gray.
248	1,305	200	3 hours	do	do	Do.
337	1,040	211	1.5 hours	do	Carrots	Do.
336	1,045	211	do	do	do	Do.

SERIES C.

122	2,060	250	2 hours 55 minutes	1.5 days	Oats	White.
234	1,650	242		About 24 hours	Carrots	Do.
335	1,170	240	1 hour	do	do	Gray coccidiosis.
249	1,185	236	4 hours	Survived	Oats	Gray.
321	1,135	246	2 hours 10 minutes	do	do	Yellow.
250	1,435	252	4 hours 35 minutes	do	do	Gray.
334	1,270	240	1 hour	do	Carrots	Do.
233	1,675	238	6 hours 10 minutes	26 hours	do	White.

TABLE 1.—*Subcutaneous injections of caffein—rabbits*—Continued.

SERIES D.

No.	Weight.	Caffein per kilo.	Appearance of symptoms in—	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
253	1,600	275	10 minutes	35 minutes	Oats	Brown and black.
252	1,335	270	30 minutes	4 hours 55 minutes	...do	Black.
327	820	292		2 hours 15 minutes	...do	White.
340	1,465	273		About 18 hours	...do	White and brown.
341	1,450	270		1 hour 25 minutes	...do	Do.
326	1,645	243	2 hours	50 hours	...do	White.
235	1,875	267		20 hours	Carrots	Gray.
316	860	267	2 hours 45 minutes	3 hours 20 minutes	Oats	Do.
395	1,410	283	3 hours	Survived		Do.
395	1,215	275	.....do	About 2 days	Oats	Do.
396	1,475	272	.....do	Survived	...do	Do.
396	1,245	275	1 hour	About 18 hours	...do	Do.
397	1,375	290		3 days	...do	Do.
398	1,570	293		5.5 hours	...do	Do.
399	1,725	300		6 hours	...do	Do.

SERIES E.

325	1,065	112	2 hours	Less than 22 hours	Oats	White female.
330	935	107	None	Survived		Gray.
329	775	103	.....do	.....do		Gray male.
320	1,040	115	2 hours	46 hours	Oats	Black male.
551	1,450	100	30 minutes	Less than 24 hours	...do	Gray female.

ADMINISTRATION BY MOUTH.

These experiments were carried out on two varieties of rabbits, the white and the gray. The diet consisted chiefly of oats, but in a few cases carrots formed the exclusive diet. Food and water were given ad libitum. A 2 per cent solution of caffein was administered through a stomach tube. Since the resistance to most drugs is commonly supposed to be greater when given by mouth than when administered by any other path, doses of 175 to 200 mg per kilo were fed in a series of preliminary experiments, all of which were performed on gray rabbits weighing from 865 to 1,135 grams, and which were fed carrots for several days previous to the experiment. Three of the rabbits survived, two without showing any symptoms; in the other case paralysis of the posterior extremities was observed five hours after he received caffein and he was found dead the next morning. Unfortunately no autopsy was performed. The low resistance to caffein of this animal was probably due to some abnormal condition which developed about the time of the experiment, since this rabbit received 325 mg of caffein per kilo two weeks previously and increased reflexes only were observed as a result of this treatment. Hence 200 mg of caffein per kilo can not be considered the toxic dose when fed by mouth. In the following experiments larger doses were therefore given.

SERIES A.

Rabbit 248. Belgian hare. Weight, 1,170 grams. Diet, oats.

November 17: 1.20 p. m., 19.5 cc 2 per cent caffein (330 mg per kilo) administered by the mouth; 4.30 p. m., somewhat hypersensitive.

November 19: No symptoms; at 9 a. m., urine collected, no reduction of Fehling's solution; rabbit survived.

Rabbit 241. White male. Weight, 1,380 grams. Diet, oats.

November 17: 1.15 p. m., 20 cc 2 per cent caffein (290 mg per kilo) administered by the mouth; 4.30 p. m., some hypersensitiveness, but no other symptoms.

November 18: 9 a. m., urine collected, no reduction of Fehling's solution; no symptoms; rabbit survived.

Rabbit 249. Belgian hare. Weight, 890 grams. Diet, oats.

November 17: 1.30 p. m., 14.5 cc 2 per cent caffein (325 mg per kilo) administered; 4.30 p. m., hypersensitiveness; no other symptoms.

November 18: 10 a. m., no symptoms; urine collected, no reduction; rabbit survived.

SERIES B.

The object of these experiments was to determine the minimum fatal dose of caffein in the two varieties of rabbits, the white and the gray. All of the animals selected were approximately of the same weight.

Rabbit 239. Belgian hare, male. Weight, 935 grams. Diet, oats.

November 19: 4 p. m., 17 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: Urine examined, no sugar found, no symptom noticed at any time after injection.

Rabbit 254. Belgian hare, female. Weight, 975 grams. Diet, oats.

November 19: 4.05 p. m., 18 cc 2 per cent caffein (369 mg per kilo) administered by mouth, followed by 10 cc of 0.9 per cent salt solution.

November 20: 9 a. m., rabbit found dead.

Rabbit 267. White. Weight, 1,050 grams. Diet, oats.

November 23: 12.10 p. m., 18 cc 2 per cent caffein (342 mg per kilo) given by mouth, followed by 18 cc salt solution; 1 p. m., increased reflexes, tremors marked but no tetanus; 1.05 p. m., rabbit stretched on abdomen, posterior extremities in extended position and paralyzed, soon after clonic spasms set in, which recurred about every minute; 1.14 p. m., tetanus and death. *Autopsy:* Liver showed fatty degeneration; slight inflammation of stomach and intestines; other organs normal.

Rabbit 268. White. Weight 1,100 grams. Diet, oats.

November 23: 20 cc 2 per cent caffein (363 mg per kilo) administered by mouth, followed by 20 cc salt solution; 1.15 p. m., somewhat hypersensitive; 4.30 p. m., tremors fairly marked, no urine passed, about 2 cc of bloody looking urine obtained from bladder, which contained albumen and a considerable amount of glycogen; rabbit died.

Rabbit 419, Belgian hare, male. Weight, 1,600 grams. Diet, oats.

September 26: 10 a. m., 28 cc 2 per cent caffein (350 mg per kilo) given by mouth; reflexes increased at 4 p. m.; 6 p. m., reflexes still increased, no other symptoms.

September 27: 9 a. m., found dead. *Autopsy:* Lungs, liver, and kidneys congested; other organs normal.

Rabbit 420. Belgian hare, male. Weight, 1,250 grams. Diet, oats.

September 26: 10 a. m., 22 cc 2 per cent caffein (352 mg per kilo) given by mouth; 11.35 a. m., convulsions; 12 noon, found dead. *Autopsy:* Liver showed very extensive coccidiosis; no other lesions.

Rabbit 421. Belgian hare, male. Weight, 1,485 grams. Diet, oats.

September 26: 10 a. m., 26 cc 2 per cent caffein (351 mg per kilo) administered by mouth; 4 p. m., reflexes increased; 6 p. m., reflexes as before, no tetanus observed.

September 27: 9 a. m., rabbit found dead. *Autopsy:* Congestion of lungs and kidneys; liver congested and slightly fatty.

Rabbit 424. White, male. Weight, 1,295 grams. Diet, oats.

September 26: 2 p. m., 19 cc 2 per cent caffein (293 mg per kilo) administered by mouth; 4 p. m., reflexes increased, no other symptoms; 6 p. m., no change since 4 p. m.

September 27: 12 noon, convulsions and death. *Autopsy:* Congestion of the lungs; no other lesions.

Rabbit 423. White, male. Weight, 1,205 grams. Diet, oats.

September 26: 2 p. m., 18 cc 2 per cent caffein administered by mouth; 4 p. m., reflexes increased, no tetanus; 6 p. m., condition unchanged since 4 p. m.

September 27: 9 a. m., found dead. *Autopsy:* Lungs liver, and kidneys congested; other organs normal.

Rabbit 422. White, male. Weight, 1,440 grams. Diet, oats.

September 26: 2 p. m., 21 cc 2 per cent caffein (291 mg per kilo) given by mouth; reflexes increased at 4 p. m.

September 27: 3 p. m., alive, no symptoms; 4 p. m., convulsions with recovery, this was soon followed by a violent attack of tetanus, which lasted about one minute and was succeeded by paralysis; rabbit died at 4.30. *Autopsy:* Liver slightly congested; a small portion of the intestine showed congestion and edema; other organs normal.

A study of these experiments shows also considerable variation in the toxicity of caffein when given by mouth. In some cases a dose of 300 mg per kilo, and even less, caused death, as in rabbits 423 and 424. In other rabbits, however, approximately the same doses of caffein produced increased reflexes only. The same symptoms were produced in Nos. 248 and 249 after the administration of 325–330 mg of caffein per kilo, while another rabbit (No. 239) survived a dose of 363 mg per kilo. That this is exceptional, however, appears from the result of the following experiments on rabbits Nos. 419, 420, and 421, all of which died after receiving 350 mg of caffein per kilo, and rabbits 267 and 268, to which doses of 363 and 342 mg, respectively, per kilo proved fatal. It will be observed further that the gray rabbits are more resistant to caffein than the white animals, as 350 mg per kilo was the smallest fatal dose for rabbits 419, 420, and 421, all of which were gray rabbits, while a dose of 290 mg per kilo was fatal for some of the white rabbits. Again, it will be noticed that of the two gray rabbits, Nos. 254 and 239, which received the largest doses in these experiments, namely, 369 and 363 mg, respectively, one survived. The largest doses given to the white rabbits were 363 and 342 mg caffein per kilo. Both of these died from the effects of the drug. It may be concluded, therefore, that the minimum toxic dose for the gray rabbit is about 325 mg of caffein per kilo, and the minimum fatal dose is at least 350 mg per kilo. It is to be remarked in this connection that post-mortem examination showed extensive coccidiosis in rabbit 420 and fatty liver in No. 421, while the macroscopical examination of the organs of Nos. 424 and 423 failed to show the presence of such abnormalities. Since, as was observed in the section on subcutaneous injection and elsewhere in this investigation, pathological changes are apt to decrease the resistance to caffein, it is quite possible that 350 mg per kilo is not the minimum fatal dose for the normal rabbit. Indeed, the experiment on rabbit 239 lends support to this view, thus furnishing additional evidence of difference in the resistance to caffein in the two varieties of rabbits.

TABLE 2.—*Administration of caffein by mouth.*

SERIES A.

Rabbit No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.	Diet.	Remarks.
	Grams.	*Mg.*				
248	1,170	330	3 hours 10 minutes	Survived	Oats	Gray.
241	1,380	290	2 hours 15 minutes	do	do	White male.
249	890	325	3 hours	do	do	Gray male.

SERIES B.

239	935	363		Survived	Oats	Gray male.
254	975	369		About 2 days	do	Gray female.
267	1,050	342	50 minutes	1 hour 4 minutes	do	White.
268	1,100	363		About 3 hours	do	Do.
419	1,600	350	6 hours	Less than 24 hours	do	Gray male.
420	1,250	352	1 hour 35 minutes	2 hours	do	Do.
421	1,485	351	6 hours	Less than 24 hours	do	Do.
424	1,295	293	2 hours	22 hours	do	White male.
423	1,205	300	do	Less than 19 hours	do	Do.
422	1,440	291	do	2½ hours	do	Do.

INJECTION INTO THE PERITONEAL CAVITY.

In a number of experiments caffein was introduced into the peritoneal cavity. Rabbits of different varieties receiving a diet of oats or carrots were employed for this purpose; food and water were given ad libitum. The minimum doses required to induce symptoms or cause death in these animals were determined; tests with caffein were also made on young rabbits in order to study the influence of age on the resistance to this substance. The results are shown in the following experiments:

SERIES A.

In this series large doses were administered, approximating 0.3 gram per kilo.

Rabbit 71. Gray female. Weight, 1,659 grams.

January 20: 2.20 p. m., 25 cc aqueous solution 2 per cent caffein (300 mg per kilo) were injected into the peritoneal cavity; 3.45 p. m., when doors of cage were opened rabbit had spasm of short duration.

January 21: Rabbit found dead.

Rabbit 72. Gray and white. Weight, 1,402 grams.

January 21: 11.17 a. m., 20.2 cc (aqueous solution) of 2 per cent caffein (300 mg per kilo) injected into peritoneal cavity from burette; 11.25 a. m., paralysis; 11.30 a. m., rabbit had convulsion when picked up from the floor, followed by several spasms later; 11.35 a. m., typical tetanus; 12.30 noon, found dead.

Rabbit 61. Black female. Weight, 2,143 grams.

January 19: 40 cc 2 per cent caffein, aqueous solution (300 mg per kilo), injected into peritoneal cavity from burette; tetanus when about 30 cc were injected; when removed from holder, repeated and violent convulsions, terminating in death.

SERIES B.

The object of these experiments was to determine the minimum lethal dose; 0.2 to 0.15 gram of caffein per kilo was injected into the rabbits of this series.

Rabbit 69. White female. Weight, 1,714 grams.

January 20: 10.15 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity. No symptoms, under observation for 45 minutes, rabbit defecated rather copiously; feces were soft; 11 a. m., 6 cc 2 per cent caffein, aqueous solution, injected into peritoneal cavity, no symptoms, under observation for 40 minutes; 11.40, 6 cc 2 per cent caffein injected into peritoneal cavity; 11.45, rabbit is restless, reflexes increased.

Rabbit 70. Gray and white female. Weight, 1,487 grams.

January 20: 1.30 p. m., 15 cc 2 per cent aqueous solution of caffein (0.2 gram per kilo) injected into the peritoneal cavity; 2.20 p. m., no symptoms.

January 30: About 2 p. m. rabbit died.

February 1: *Autopsy:* Cirrhosis of the liver; enteritis of small intestines; stomach and kidneys normal.

Rabbit 93. Maltese, male. Weight, 1,197 grams.

March 2: 11.30 a. m., 12 cc of 2 per cent caffein (200 mg per kilo) injected into peritoneal cavity; 11.35 a. m., while being released from holder, tetanus occurred, repeated attacks later, clonic convulsions with tonic rigidity of posterior extremities during the attacks as well as during intervals, anterior extremities were relaxed during the intervals between the attacks, opisthotonos of cervical region but kyphosis in lumbar region were observed, no salivation nor dilatation of the pupils; 2 p. m., rabbit died.

Rabbit 92. Yellow female. Weight, 1,388 grams.

February 25, 4.15 p. m., 14 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4.20 p. m., restlessness and increased reflexes, rabbit found stretched out in cage, but raised himself on his legs again; 4.45, general tremor when touched.

February 26: 9 a. m., rabbit found dead. *Autopsy:* Hemorrhage into abdominal muscles at site of injection; hemorrhage also in walls of stomach opposite similar spot in abdominal wall.

Rabbit 309. Belgian hare, female. Weight, 1,500 grams. Diet, oats.

March 2: 2.05 p. m., 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.25 p. m., found dead, no urine found in bladder.

Rabbit 307. Belgian hare, female. Weight, 1,320 grams. Diet, oats.

March 2: 12 noon, urine obtained from bladder, clear amber colored, no albumin, no reduction; 12.06 p. m., 10 cc of 2 per cent caffein (0.151 gram per kilo) injected into peritoneal cavity; 1.30 p. m., rabbit placed on floor, runs around but anterior and posterior extremities soon extended, in tonic condition; 2.25 p. m., reflexes increased, paralysis of extremities, dyspnœa; 4.50 p. m., about 100 cc urine collected, no albumin, reduction of Fehling's solution moderate.

March 3: 9.30 a. m., posterior extremities extended and rigid, anterior extremities paralyzed, respiration less frequent and deeper than normal. Rabbit died at 11.50 a. m.; urine collected since 4.50 p. m. previous day gave very heavy reduction of Fehling's solution. *Autopsy:* Animal in good condition; in the left axillary region was observed a hemorrhage into the subcutaneous and muscular tissue of that region. The ventral portion of the large colon, in contact with the ventral abdominal wall, showed a hemorrhagic area about one-half inch in length, such as might be produced by a puncture or bruise of the colon through the abdominal wall. A small portion of the small intestine adjacent to the colon was affected in a similar manner. All internal organs were apparently normal.

Rabbit 308. Belgian hare, female. Weight, 1,350 grams. Diet, oats.

March 2: 11.45 a. m., urine obtained from bladder, no albumen, no reduction; 11.50 a. m., 10 cc 2 per cent caffein (0.15 gram per kilo) injected into peritoneal cavity; 1.30 p. m., not very active, no abnormal symptoms otherwise; 3. 30 p. m., rabbit looked depressed, made very little attempt to move about, remained in one position most of the time when placed on floor; 4.30 p. m., 180 cc urine collected, no albumen, reduction of Fehling's solution moderate.

March 3: 9.30 a. m., rabbit looks normal, is able to walk but is easily fatigued when made to walk about or when placed on his side, followed by paralysis of anterior extremities, posterior extremities apparently normal, about 90 cc of urine collected at noon was free from albumen, did not reduce Fehling's solution.

March 4: 11 a. m., lying on his side in cage, anterior extremities limp, posterior extremities extended and rigid, is in dying condition.

March 5: 9 a. m., found dead. *Autopsy:* Liver engorged; spleen congested, but not enlarged; kidneys, some congestion in cortex; stomach filled, mucosa thickened and easily pulled off; petechial hemorrhages on serosa of colon.

SERIES C.

The experiments of this series were made to determine the minimum toxic dose.

Rabbit 295. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 10.40 a. m., 6 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; about 2 cc of urine obtained before injecting caffein; 2 p. m, 100 cc urine, bloody in appearance, collected, a moderate quantity of albumen present, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well.

Rabbit 293. Belgian hare, female. Weight, 1,605 grams. Diet, carrots.

March 1: Urine from bladder clear, alkaline; 11.55 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; 3 p. m., 90 cc urine normal in color collected, no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 1.15 p. m., rabbit looks normal.

Rabbit 292. Belgian hare, male. Weight, 1,595 grams Diet, carrots.

March 1: 10.10 a. m., 8 cc 2 per cent caffein (0.1 gram per kilo) solution injected into peritoneal cavity; 10.40 a. m., rabbit urinated, reflexes increased, but no other symptoms; 10.50 a. m., no urine obtained from bladder; 2 p. m., 105 cc of clear pale urine collected; no albumen, no reduction; 3.40 p. m., no symptoms.

March 4: 2 p. m., rabbit looks well, urine collected, did not contain sugar.

Rabbit 298. Belgian hare, female. Weight, 1,205 grams. Diet, carrots.

March 1: 4.06 p. m., 7.5 cc 2 per cent caffein solution (0.125 gram per kilo) injected into peritoneal cavity, urine obtained from bladder immediately after injection, no albumen, no reduction; 5.30 p. m., reflexes increased, rabbit was able to run around, but became paralyzed soon; 5.40 p. m., rabbit is again able to run around.

March 3: 10 a. m., anterior extremities paralyzed, is able to use posterior extremities.

March 4: 1 p. m., rabbit looks normal.

Rabbit 223. Belgian hare, male. Weight, 1,165 grams. Diet, carrots.

March 1: 3.50 p. m., urine obtained from bladder clear, amber colored, no albumen, no sugar; 3.52 p. m., 7.5 cc 2 per cent caffein (125 mg per kilo) injected into peritoneal cavity; 5.40 p. m., rabbit makes little attempt to run when put on the floor, weakness of extremities marked.

March 4: 1.15 p. m., rabbit normal.

SERIES D.

The object of the experiments of this series was to study the effect of age on the resistance to caffein. Half-grown rabbits were, therefore, used in the following experiments.

Rabbit 310. Belgian hare, female. Weight, 880 grams. Diet, oats.

March 2: 3.25 p. m., 9 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity.

March 3: 9.30 a. m., no symptoms, rabbit looks normal.

March 4: 11 a. m., posterior extremities abducted, walked when placed on the floor, made no attempt to change attitude when placed on its side, remained some time in this position.

March 5: 9 a. m., found dead. *Autopsy:* Liver showed areas of degeneration; kidneys congestion and petechial hemorrhage on cortex; small and large intestines, inflammation marked; bladder distended.

Rabbit 75. Gray and white, female. Weight, 842 grams.

January 25: 3 p. m., 8.5 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 3.15 p. m., anterior extremities weak and reflexes increased.

January 27: Rabbit paralyzed but is able to turn over when placed on back.

Rabbit 74. Gray and white, female. Weight, 692 grams.

January 25: 3 p. m., 7 cc 2 per cent caffein (0.2 gram per kilo) solution injected into peritoneal cavity; 3.15 p. m., reflexes increased and anterior extremities paralyzed.

January 27: Rabbit recovered and is able to walk about in the room.

Rabbit 312, maltese, female. Weight, 740 grams. *Diet, oats.*

March 3: 11.47 a. m., urine obtained from bladder, appearance normal, no albumen, no reduction of Fehling's solution; 11.50 a. m., 7.5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m. anterior extremities paralyzed, posterior extremities rigid and extended; 5 p. m. (about), rabbit died.

Rabbit 311. Belgian hare, female. Weight, 650 grams. Diet, oats.

March 3: 11.26 a. m., urine obtained from bladder normal in appearance, albumen considerable, reduction of Fehling's solution none; 11.27 a. m., 6 cc 2 per cent caffein solution (0.2 gram per kilo) injected into peritoneal cavity; 2.30 p. m., rabbit seemed to be normal, no symptoms had developed; urine collected contained a large amount of sugar, reduction was very heavy, but no albumen was found.

March 4: 11 a. m., condition good, moves about when put on floor; gait, normal.

Rabbit 78. Yellow and white. Weight 659 grams.

January 26: 1.30 p. m., 8.5 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity, under observation the rest of the afternoon, no symptoms.

January 27: 4 p. m., no symptoms developed.

Rabbit 317. Belgian hare, female. Weight 635 grams. Diet oats.

March 15: 10.35 a. m., 8 cc 2 per cent caffein (0. 252 gram per kilo) injected into peritoneal cavity; 12 noon, marked abduction of hind legs, was unable to walk after a little exertion, rabbit died between 12.30 and 12.50 p. m. *Autopsy.* Right lung hepatized and showed adhesions to costal and mediastinal pleura; liver studded with nodules of coccidiosis; spleen congested; stomach filled, mucosa normal; intestines injected; colon hemorrhagic on serosa in ventral region, near point of injection; kidneys normal.

Rabbit 323. White, female. Weight 820 grams. Diet oats.

March 15: 10.45 a. m., 10 cc 2 per cent caffein (250 mg per kilo) injected into peritoneal cavity; 12 noon, reflexes increased, hind legs abducted but is able to walk, symptoms are mild; 1.40 p. m., tremors, weakness, and abduction of head and legs much more marked than at 12 noon.

March 16: Condition good.

March 17: Condition good, recovery apparently complete.

Since the experiments of Series A, which were intended as preliminary tests, have shown that 0.3 gram of caffein per kilo when introduced into the peritoneal cavity is rapidly absorbed and is fatal, much smaller doses were employed in subsequent trials with the drug. This is shown in series B, which may be divided into two groups. Group I, consisting of rabbits 69, 70, 92, 93, and 309, which received 0.2 gram of caffein per kilo, and Group II, Nos. 307 and 308, into which 0.15 gram of caffein per kilo was injected. Three rabbits of Group I (Nos. 92, 93, 309) died from the effects of caffein; rabbit 309 twenty minutes after injection, and rabbits Nos. 92 and 93, twenty hours and two and one-half hours, respectively, after the administration of caffein. In both of these rabbits symptoms appeared within five minutes after the injections were made. Rabbits 69 and 70, it will be noticed, survived the same amount of caffein in proportion to body weight as was given to the other members of this group. Increased peristalsis and the distribution of the dose may account for the greater resistance of rabbit No. 69. The case of rabbit No. 70 is evidently one of exceptional resistance to caffein, since both the rabbits of Group II died from the effects of a much smaller dose, namely, 0.15 gram of caffein per kilo. Moreover, macroscopical examination at the autopsy of Nos. 307 and 308 failed to show any lesions which might tend to lessen the resistance to caffein.

That a dose of 0.15 gram per kilo is therefore in all probability the minimum fatal dose for the rabbit when injected into the peritoneal cavity appears from the results of the experiments in series C, in which smaller doses, 0.125 gram of caffein per kilo caused mild symptoms only, while 0.1 gram per kilo rarely induced any symptoms. It may be remarked that the rabbits of series C were fed carrots while rabbits Nos. 307 and 308 received oats. Their resistance to caffein may be different, but, as was pointed out in the earlier part of this investigation, diet does not seem to influence the toxicity of the single dose of caffein. Doses of 150 and of 100 to 125 mg per kilo, when injected into the peritoneal cavity, may be considered, respectively, as the minimum fatal and minimum toxic doses for the gray rabbit. Analysis of the experiments in series D shows much greater resistance to caffein than in the other rabbits which received it intraperitoneally. Thus, after the administration of 0.2 gram per kilo to each of five rabbits, no effect was observed in two cases (Nos. 310, 311), while in two others (Nos. 74, 75) symptoms developed, but they survived. Only one rabbit, No. 312, died from the effects of this dose; the autopsy showed the presence of degeneration of the liver and petechial hemorrhages on the cortex of the kidneys in the case of No. 310, which was probably the cause of death rather than the caffein.

Two decigrams of caffein can not be considered, therefore, the fatal dose for rabbits. This is further corroborated by the results obtained in experiments with larger doses. Rabbit 78, which received 257 mg per kilo, failed to show any symptoms. The same amount in proportion to body weight in No. 323 caused mild symptoms only, while the rapid death of rabbit No. 317 after the same dose of caffein may be explained by the lesion found at autopsy, thus affording additional evidence that disease may decrease the resistance to caffein. It will be observed that all the members of this series were young rabbits and, as will be shown later, young animals of other species are likewise more resistant to caffein than adult animals. Similar results were obtained by von Anrep, who observed that atropin is less toxic in young than in full-grown animals.

Observations were also made on the diuretic effect of caffein when injected into the peritoneal cavity. The results shown in the following table indicates the stimulating effect on renal secretion whether the diet consisted of oats or of carrots. The urine of some rabbits contained moderate amounts of sugar after from 0.2 to 0.15 gram of caffein per kilo was given; albumen was observed in one case, but in none of the others. In rabbit No. 311 albumin was found before the injection of caffein, but none in the urine which was collected three hours after caffein was injected.

Effect of caffein on renal secretion.

No.	Weight.	Caffein per kilo.	Urine.	Time.	Diet.
	Grams.	*Gram.*	cc.		
307	1.320	0.150	100	4.5 hours	Oats.
308	1,305	.150	180	do	Do.
295	1,205	.100	100	2 hours 20 minutes.	Carrots.
293	1,605	.100	90	3 hours	Do.
292	1,595	.100	105	4 hours	Do.

NOTE.—The amount of urine secreted in three hours by control rabbits, on a carrot diet, varied between 35 and 50 cc, the average weight of the animals being a little above 1,600 grams. The secretion of urine on an oat diet was much less for an equal period of time.

TABLE 3.—*Intraperitoneal injections.*

SERIES A.

No.	Weight.	Caffein per kilo.	Time of appearance of symptoms.	Duration of life.	Remarks.
	Grams.	*Gram.*			
71	1,659	0.3	85 minutes	24 hours	Gray.
61	2,143	.3		At the end of injection	Black.
72	1,402	.3	8 minutes	1.25 hour	Gray and white.
SERIES B, GROUP I.					
70	1,467	0.2		10 days	Gray and white.
93	1,492	.2	5 minutes	2.5 hours	Maltese; given second dose after 3 days, died 2.5 hours later.
69	1,492	.2	About 5 minutes	Survived	White.
92	1,388	.2	5 minutes	24 hours	Yellow.
309	1,500	.2		20 minutes	Belgian; oats.
SERIES B, GROUP II.					
308	1,350	0.15	3 hours and 40 minutes.	About 2.5 days	Belgian; oats.
307	1,320	.15	1 hour 24 minutes	24 hours	Do.
SERIES C.					
223	1,165	0.125	2 hours	Survived	Belgian; carrots.
293	1,605	.1		do	Do.
295	1,205	.1		do	Do.
292	1,595	.1		do	Do.
298	1,205	.125	1.5 hours	do	Do.
SERIES D.					
310	880	0.2	2 days [1]	About 2.5 days [1]	Belgian; oats.
311	650	.2		Survived	Do.
312	740	.2	40 minutes	4.5 hours	Maltese; oats.
78	659	.257	15 minutes	Survived	Yellow and white; oats.
75	842	.2	do	do	Gray and white.
74	692	.2	do	do	
317	635	.252	1 hour 25 minutes	About 2 hours	Belgian; oats.
323	820	.25	1 hour 15 minutes	Survived	White; oats.

[1] Not due to caffein.

INTRAMUSCULAR INJECTION.

Well-fed rabbits, which received a diet exclusively of oats, were used for these experiments. The injections were made into the lumbar or into the gluteal muscles.

SERIES A.

In this series the caffein was injected into the gluteal muscles.

Rabbit 284. Brown and white, female. Weight, 1,100 grams.

December 14: 2 p. m., 11 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), under observation until 5 p. m., had frequent convulsions; at 5 p. m. in a comatose condition. Rabbit was found dead the next morning.

Rabbit 286, white and black, female. Weight, 1,315 grams.

December 15: 2.30 p. m., 13 cc 2 per cent caffein injected into the gluteal muscles (0.1977 gram per kilo), tremors and increased reflexes observed during the next two hours, but no other symptoms.

December 17: Rabbit alive.

Rabbit 285, yellow and white, female. Weight, 1,385 grams.

December 14: 10.15 a. m., 14 cc 2 per cent caffein injected into the gluteal muscles (0.2 gram per kilo), general tremors, but no convulsions observed. Rabbit survived.

December 17: Rabbit still alive.

Rabbit 287. Belgian hare, female. Weight, 1,140 grams.

December 15: 2.15 p. m., 11 cc of 2 per cent caffein injected into the gluteal muscles; 2.30 p. m., tonic contractions of posterior limbs. Paralysis and death at 2.40 p. m.

SERIES B.

In series B the caffein was injected into the lumbar muscles.

Rabbit 307. Belgian hare, female. Weight, 1,175 grams.

February 16: 11.05 a. m., 8 cc 2 per cent caffein injected (0.136 gram per kilo) into the lumbar muscles; under observation until 4 p. m., no symptoms; 4 p. m., allowed to walk on the floor; after walking a short distance loss of coordination and paralysis of posterior extremities; 5.20 p. m., found dead.

Rabbit 306. Belgian hare, female. Weight, 1,860 grams.

February 16: 11 a. m., 12.5 cc 2 per cent caffein injected into the lumbar muscles; 12 noon, no symptoms; 2 p. m., walked about 10 feet, exhaustion and paralysis; 3 p. m. found dead.

Rabbit 181. Belgian hare. Weight, 1,230 grams. (Was experimented on some time previously.)

February 16: 10.55 a. m., 8 cc 2 per cent caffein injected into the lumbar muscles; (0.130 gram per kilo); 12 noon, no symptoms; 2 p. m., no symptoms; 3 p. m., put on the floor, walked about 10 feet and was exhausted, posterior extremities paralyzed; 4 p. m., found dead.

SERIES C.

In the fall of the same year additional experiments were carried out with doses ranging from 100 to 200 milligrams of caffein per kilo, which were injected into the lumbar muscles. The results are given in the following abbreviated protocols:

Rabbit 425. Belgian hare. Weight 1,520 grams.

September 27: 10.30 a. m., 7.5 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1,620 grams; 2.50 p. m., 10 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.

October 13: Weight, 1,520 grams; 10.30 a. m., 10 cc 2 per cent caffein (131 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes much increased.

October 14: Alive, no symptoms.

Rabbit 426. Belgian hare, female. Weight, 1,425 grams.

September 27: 7 cc 2 per cent caffein injected into the lumbar muscles at 10.30 a. m.; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1,425 grams; 2.55 p. m., 9 cc 2 per cent caffein injected into lumbar muscles; 3.05 p. m., reflexes increased.

October 13: Weight, 1,405 grams; 10.30 a. m., 10 cc 2 per cent caffein (142 mg per kilo) injected; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.

October 14: Rabbit alive, no symptoms.

Rabbit 427. Belgian hare, female. Weight, 1,780 grams.

September 27: 9 cc 2 per cent caffein injected into the lumbar muscles; 2 p. m., reflexes increased.

September 28: Rabbit normal.

October 5: Weight, 1, 850 grams; 3 p. m., 11.5 cc 2 per cent caffein injected into lumbar muscles; 3.10 p. m., reflexes increased.

October 13: Weight, 1,830 grams; 10.40 a. m., 14 cc 2 per cent caffein (153 mg per kilo) injected into lumbar muscles; 11 a. m., no symptoms; 11.30 a. m., reflexes increased.

October 14: Rabbit alive, no symptoms.

Rabbit 453. Belgian hare, male. Weight, 1,160 grams.

October 12: 3.45 p. m., 11.5 cc 2 per cent caffein in aqueous solution injected into lumbar muscles; 4.15 p. m., reflexes increased; 4.30 p. m., paralyzed.

October 13: 9 a. m., found dead. *Autopsy:* Gastric mucosa hemorrhagic; liver darkened; other organs normal.

Rabbit 455. Belgian hare, gray, female. Weight, 1,185 grams.

October 12: 3.30 p. m., 11.5 cc 2 per cent caffein injected into the lumbar muscles; 4 p. m., reflexes increased.

October 13: Rabbit weighed 1,070 grams, no symptom of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., jumped off the table, had attack of convulsions and died. *Autopsy:* Findings same as in No. 453.

Rabbit 428. Belgian hare, gray, male. Weight, 1,650 grams.

October 5: 4 p. m., 14.8 cc 2 per cent caffein (0.18 gram per kilo) injected into the lumbar muscles.

October 6: Found dead.

Rabbit 429. Belgian hare, male. Weight, 1,340 grams.

October 5: 4 p. m., 13.5 cc 2 per cent caffein (0.2 gram per kilo) injected into lumbar muscles.

October 8: Rabbit found dead.

SERIES D.

Further experiments making injections into both the lumbar and the gluteal muscles, were made in this series.

Rabbit 577. Gray male. Weight, 1,380 grams.

February 14: 3 p. m. 14 cc 2 per cent caffein injected into the gluteal muscles of the right side; 3.10 p. m., restless, jumped off the table and walked about, reflexes increased; 3.45 p. m., passed 30 cc clear, straw-colored urine; 4.45 p. m., allowed to walk about, ran across the room, about 20 feet, looked tired, stretched himself out on the floor, then raised himself and walked about showing no disturbance of gait.

February 15: 9 a. m., found dead.

Rabbit 578. Gray, female. Weight, 1,670 grams.

February 14: 3.05 p. m., 18 cc 2 per cent caffein solution injected into the gluteal muscles of the right side; 3.15 reflexes increased, but not restless; 5 p. m., allowed to walk about, no symptoms observed.

February 15: Found dead.

Rabbit 579. White and gray, male. Weight, 1,490 grams.

February 14: 3.15 p. m., 15 cc 2 per cent caffein solution injected into the gluteal muscles of the right side, put in cage; 3.30 p. m., reflexes increased; 5 p. m., taken out of cage and allowed to walk across the room, no special symptoms noticed.

February 23: Still alive.

Rabbit 580. Gray male. Weight, 1,510 grams.

February 14: 3.35 p. m., 15 cc 2 per cent caffein solution injected into lumbar muscles.

February 23: Still alive, in good condition.

Rabbit 581. Gray female. Weight, 1,680 grams.

February 14: 3.45 p. m., 17 cc 2 per cent caffein solution injected into the lumbar muscles of the right side; 4 p. m., reflexes increased; 4.15 p. m., jumped off the table and had wild convulsions, became very restless, walked about the laboratory; 4.25 p. m., had convulsions occasionally; 4.30 p. m., extremities extended and quite rigid; 4.35 p. m., convulsions and death.

Rabbit 582. Gray male. Weight, 1,870 grams.

February 14: 4.15 p. m., 18 cc 2 per cent caffein solution injected into the lumbar muscles of right side; 5 p. m., reflexes increased; walked about in the room, then rested; 5.15 p. m., had short spasm when handled.

February 23: Alive; good condition.

The data presented in these experiments show that the toxicity of caffein when injected into the muscles of the lumbar regions is the same as when injected into the gluteal muscles. The rabbits of series A received approximately 0.2 gram caffein per kilo and two died as a result of this treatment. The other two survived but symptoms of caffein intoxication were observed.

In series B smaller doses proved fatal, from which it would appear that caffein is more toxic when injected into the lumbar muscles. Further observations, however, failed to corroborate the results obtained in this series. Thus, in series C, 130 to 150 mg of caffein per kilo injected into the lumbar muscles produced mild symptoms only. Experiments with larger doses showed that 0.180 gram caffein per kilo may cause death. It will be noticed, on the other hand, that rabbit No. 455 survived a dose of 0.2 gram per kilo. New experiments were therefore carried out in which the same amounts of caffein in proportion to the weight of the animals were injected into the lumbar muscles as into the gluteal muscles. As shown in the experiments of series D, one rabbit (No. 581) died shortly after caffein was injected into the lumbar muscles; two recovered. Two of the three which received injections into the gluteal muscles were found dead the next day; one recovered. Post-mortem examination failed to indicate the presence of any abnormalities. The rate of absorption of caffein from the gluteal and from the lumbar muscles seems to be, therefore, the same, or not to differ very much. The observations of Auer and Meltzer[7] are of interest in this connection. According to their investigations adrenalin is more rapidly absorbed from the lumbar than from the gluteal muscles. This is in all probability due to the greater delicacy of the test they employed (since they judged the rate of absorption by the effect of adrenalin on blood pressure) as well as to the much greater activity of the substance.

TABLE 4.—*Intramuscular injections.*

No.	Weight.	Caffein per kilo.	Symptoms after—	Duration of life.	Site of injection.	Remarks.
SERIES A.						
	Grams.	*Gram.*				
284	1,100	0.200	3 hours	Less than 20 hours	Gluteal	White and brown female.
286	1,315	.1977	2 hours	Survived	do	White and black female.
285	1,385	.200	Present	do	do	Yellow and white female.
287	1,140	.210	15 minutes	25 minutes	do	Gray female.
SERIES B.						
307	1,175	0.136	5 hours	6 hours, 20 minutes	Lumbar	Gray female.
306	1,860	.134	3 hours	4 hours	do	Do.
181	1,230	.130	4 hours	5 hours	do	Gray.
SERIES C.						
425	1,520	0.131	1 hour	Survived	Lumbar	Gray.
426	1,405	.142	30 minutes	do	do	Gray female.
427	1,830	.153	50 minutes	do	do	Do.
453	1,160	.200	30 minutes	Less than 20 hours	do	Gray male.
455	1,185	.200	do	Survived	do	Gray female.
428	1,650	.180		Less than 20 hours	do	Gray male.
429	1,340	.200		do	do	Do.
SERIES D.						
577	1,380	0.200	10 minutes	Less than 18 hours	Gluteal	Gray male.
578	1,670	.210	do	do	do	Do.
579	1,490	.200	15 minutes	Survived	do	White and gray male.
580	1,510	.200		do	Lumbar	Gray male.
581	1,680	.200	15 minutes	50 minutes	do	Do.
582	1,870	.192	45 minutes	Survived	do	Do.

Examination of Table 4 shows that 14 rabbits received from 180 to 210 mg caffein per kilo. The appearance of symptoms in these rabbits varied considerably. In some increased reflexes could be noticed in 10 to 15 minutes after the injection of caffein; in others it was delayed 2 or 3 hours. It might be added that the onset of symptoms occurred in many cases very soon after the administration of the drug—on an average about 10 to 30 minutes after the drug was injected. After smaller doses were administered by injection into the lumbar muscles the appearance of symptoms was delayed several hours in some cases. The duration of life in these 14 rabbits varied considerably. Eight of them died within 1 to 20 hours; six survived. About 0.2 gram caffein per kilo may be regarded as the minimum fatal dose, while the minimum toxic dose is somewhere between 130 and 150 mg per kilo.

INTRAVENOUS INJECTION.

These experiments were carried out on well-fed, full-grown gray rabbits. The diet for several days preceding the experiments consisted of oats or carrots, which were given ad libitum. The injections were made into the ear veins from a burette or by means of a syringe, the temperature of the caffein solution being about 40° C. Attention was also directed to the effect of the rate of injection and of the concentration on the toxicity. The minimum toxic as well as lethal doses were determined as shown in the following experiments.

SERIES A.

In these experiments the rate of injection was about 1 cc of 2 per cent caffein solution per minute.

Rabbit 194. White, female. Weight, 1,310 grams.

October 19: Injected 7.5 cc 2 per cent solution caffein (115 mg per kilo) into the ear vein. Rabbit showed stiffness; paralysis of extremities appeared soon after.[a] Rabbit survived.

Rabbit 556. Gray, female. Weight, 1,635 grams.

January 31: 2 p. m., 11 cc 2 per cent caffein (134 mg per kilo) injected into ear vein, in about 11 minutes; 2.10 p. m., convulsions, rabbit remained lying on its side; during the rest of the hour it had convulsions occasionally; 3.20 p. m., convulsions and died. Rabbit did not urinate after the injection of caffein.

Rabbit 557. Gray, female. Weight, 1,580 grams.

January 31: 2.30 to 2.37 p. m., 7 cc 2 per cent caffein injected from the burette at the rate of 1 cc per minute; 2.37 p. m., flow of liquid ceased, veins were engorged and bled freely, injection was continued by means of a syringe; 2 cc 2 per cent caffein injected in two minutes; injections discontinued as convulsions appeared; 2.50 p. m., rabbit raised itself but fell over; 3.10 p. m., rabbit assumed normal attitude, walked about the floor without manifesting any signs of the effects of caffein; 4.30 p. m., walked about, gait normal, condition seemed to be good.

February 1: 2 p. m., condition good, appetite good, total amount of caffein injected, 9 cc 2 per cent solution, or 114 mg per kilo.

Rabbit 558. Gray, female. Weight, 1,590 grams.

January 31: 3 p. m., given 8 cc 2 per cent caffein in eight minutes; 3.10 p. m., violent convulsions; 3.20 p. m., rabbit was stretched out on his abdomen, extremities extended, urinated; 4.30 p. m., looked normal; was able to walk about.

February 1: 2 p. m., condition good, appetite good.

Rabbit 292. Belgian hare, male. Weight, 1,770 grams.

February 18: 4.26 to 4.39 p. m., 12.5 cc warm caffein solution (0.141 gram per kilo) injected into ear vein, convulsion followed when this quantity was injected, tonic rigidity of limbs followed soon after; 4.52 p. m., condition unchanged, rabbit on floor, limbs stretched out, and lying on abdomen.

Rabbit 294. Belgian hare, female. Weight, 1,350 grams. Carrot diet for about 10 days before the experiment.

February 19: 12.20 p. m., 5 cc 2 per cent caffein (74 mg per kilo) injected into ear vein in five minutes, edema of the ear, other ear used, 3.5 cc injected in 10 minutes, repeated convulsions; 1.25 p. m., rabbit still alive, frequent attacks of convulsions; 2.30 p. m., found dead. Total amount injected in 15 minutes, 8.5 cc, or 0.126 gram per kilo.

It will be observed in the preceding experiments that symptoms of severe intoxication were present in all of the six rabbits, but only two of these (Nos. 294 and 556) died from the effects of caffein. Of those which survived, three received doses of

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[a] Time of injection inadvertently omitted, but was probably not slower than in the other cases of this series.

100 to 114 mg caffein per kilo, and another (No. 292) received 141 mg of caffein per kilo. The death of rabbits Nos. 294 and 556 may be regarded therefore as a case of exceptionally low resistance to caffein.

SERIES B.

Doses of 160 to 200 mg caffein per kilo were employed in these experiments. The rate of injection was 1 cc per minute, with the exception of Experiment 254, in which 10.8 cc 2 per cent caffein were introduced in 17 minutes and 25 seconds.

Rabbit 562. Gray female. Weight, 1,650 grams. Diet, oats.

February 1: Injection began at 3 p. m., injected 10 cc in 12 minutes; 3.01 p. m. to 3.09 p. m., 3 cc injected, convulsions; 3.09 p. m. to 3.14 p. m., 3 cc injected, followed by violent convulsions, marked opisthotonos; 4.30 p. m., rabbit died; total quantity injected, 16 cc.

Rabbit 561. Gray female. Weight, 1,450 grams. Diet, oats.

February 1: Injection began at 11.40 a. m.; 11.48, rabbit struggled, 7 cc 2 per cent caffein injected; 11.50, convulsions, 10 cc 2 per cent caffein total amount injected; 11.55 a. m., injections stopped; injections resumed 11.58, violent convulsions, injections discontinued, total quantity received, 14.5 cc 2 per cent caffein solution; 1.30 p. m., found dead, did not urinate, 25 cc urine found in the bladder.

Rabbit 560. Gray male. Weight, 1,620 grams. Diet, oats.

February 1: Injection began 11 a. m.; 11.10 a. m., 7 cc 2 per cent caffein injected, rabbit struggled; 1 cc was injected during the next three minutes, rabbit struggled but there were no convulsions, injection stopped; resumed at 11.15 a. m. and continued 10 minutes, 8 cc 2 per cent caffein introduced during this time; total amount caffein injected, 16 cc; reflexes markedly increased; 12 noon, tetanic convulsions off and on until 2 p. m., then remained stretched out on abdomen, extremities extended.

February 2: 9 a. m., found dead.

Rabbit 559. Gray female. Weight, 1,875 grams. Diet, oats.

January 31: 4 p. m., convulsions after injection of 9 cc 2 per cent caffein in 14 minutes; 4.08 p. m., convulsions after injection of 7 cc caffein in 8 minues; 4.10 to 4.12 p. m., injected 2 cc more, rabbit lying stretched out on abdomen, extremities extended; total amount of caffein injected, 18 cc (190 mg per kilo).

February 1: 2 p. m., condition good, walked about, appetite good, passed 155 cc dark, reddish-brown urine since 5.30 p. m. previous day.

Rabbit 279. Gray and white female. Weight, 1,320 grams.

February 24: 10.09 a. m., 6 cc 2 per cent caffein passed rapidly into jugular vein; 10.15 a. m., involuntary twitching of muscles of legs, but no other symptoms; 10.23 to 10.26, 3 cc of 2 per cent caffein injected; 10.27 to 10.28, 2 cc 2 per cent caffein injected, convulsions; 10.29, convulsions stopped; 10.32, convulsions; 11 a. m., rabbit lying on its side, anterior extremities paralyzed, posterior extremities contracted, no clonic convulsions, breathed deeper and more slowly than normal; 11.10 a. m., rabbit died, had no convulsions immediately before death; amount of caffein injected, 11 cc 2 per cent solution, or 0.166 gram per kilo.

Rabbit 254. Belgian hare, female. Weight, 1,285 grams. Diet, oats.

November 12: 1.30⅓ to 1.47¾ p. m., received 10.8 cc 2 per cent caffein from burette into ear vein, after injection of 6.2 cc dyspnœa, 6.7 cc struggling, convulsions; at 1.50½ p. m., released from holder, paralysis especially marked in the anterior extremities; 1.50 p. m., recovered, survived; total amount injected, 10.8 cc 2 per cent caffein in 17 minutes and 25 seconds, or 0.16 gram caffein per kilo.

Rabbit 255. Belgian hare, male. Weight, 1,105 grams. Diet, oats.

November 12: 2.31¾ to 2.35¼ p. m., received 3.7 cc; from 2.37⅙ to 2.46⅙ p. m., 5 cc injected; after injection of 6.1 cc convulsions followed by dyspnœa, then continuous struggling; when 8.3 cc were injected rabbit had another convulsion; 2.47 p. m., tonic contraction of anterior extremities; amount injected, 8.7 cc (158 mg per kilo) in 15 minutes and 35 seconds.

Rabbit 567. Gray female. Diet, oats.

February 6: Injection began at 4.11 p. m.; 4.18, convulsions after injection of 5 cc 2 per cent caffein; 4.21, convulsion after total injection of 8 cc; 4.24 p. m., injection resumed and 2 cc more introduced; 4.28 p. m., convulsions, injected 2 cc more; total caffein injected, 12 cc, or 162 mg per kilo; 4.40 p. m., rabbit paralyzed in posterior extremities; 5 p. m., found dead.

In the eight experiments comprising series B rabbits Nos. 567, 254, 279, and 255, which may be designated as Group II, received doses of 162, 160, 166, and 158 mg, respectively. Nos. 562, 561, 560, and 559, which may be designated as Group I, received about 200 mg caffein per kilo. In Group II, which received the smaller doses, one (No. 254) survived. This may be regarded as exceptional, since, as was shown in the experiments of the preceding series, even smaller doses may be fatal. About 160 mg per kilo is, therefore, the smallest surely fatal dose. This might be regarded as a contradiction of the results obtained for rabbit No. 559, but it will be noticed that in this case diuresis was very marked. The results of experiments Nos. 294 and 255 are of interest in this connection, since they indicate that a moderate difference in the rate of injection is without any effect on the toxicity of caffein. The greater resistance to caffein of rabbit No. 559 is in all probability due, therefore, to increased diuresis.

SERIES C.

In these experiments the minimum toxic dose was determined. The conditions were the same as in the experiments of the other series.

Rabbit 293. Belgian hare, female. Weight, 1,610 grams. Diet, oats.

February 18: 3.40 to 3.43 p. m., 4 cc 2 per cent warm caffein solution injected into ear vein, convulsions when 3 cc were injected, repeated attacks; 4 p. m., raised itself on legs, but fell over immediately and lay stretched on abdomen.

February 19: 9 a. m., rabbit looked normal, apparently recovered.

Rabbit 227. White male. Weight, 2,320 grams.

October 26: 3.29½ to 3.37½ p. m., injected into ear from burette 6.7 cc 2 per cent caffein, no symptoms; experiment discontinued; survived.

Rabbit 563. Gray female. Weight, 1,650 grams. Diet, oats.

February 6: Injection began at 1.02 p. m., injected 3.5 cc 2 per cent caffein (42 mg per kilo) in four minutes, 0.6 cc more within the next two and one-half minutes, total amount injected 4.1 cc; 1.10 p. m., hypersensitive, some disturbance of muscular coordination; restlessness; 1.35. p m., reflexes decreased, urinated and walked about, gait normal. Under observation for several days; no symptoms noted.

Rabbit 564. Gray female. Weight, 1,515 grams.

February 6: Injection began at 1.26 p. m., 3.5 cc 2 per cent caffein (46 mg per kilo) injected at the rate of 1 cc per minute; 1.30 p. m., reflexes increased; 1.34 p. m., marked paresis of the extremities, rabbit stretched out on abdomen, legs abducted and partly extended, able to hop about but gait disturbed, no untoward symptoms noticed, under observation for several days after experiment.

Rabbit 565. Gray female. Weight, 1,545 grams. Diet, oats.

February 6: Started to inject at 3.40 p. m., received 2.5 cc 2 per cent caffein intravenously in two minutes or 32 mg per kilo, under observation all afternoon, no symptoms.

Rabbit 566. Gray female. Weight, 1,900 grams. Diet oats.

February 6: Injection began at 3.05 p. m., received 3 cc 2 per cent caffein intravenously in three minutes or 31 mg per kilo, no symptoms observed.

These experiments show that a dose of about 50 mg per kilo when injected intravenously produces mild symptoms, such as increased reflexes. In the four experiments with this amount of caffein these effects were observed in each case. In the experiments in which smaller quantities, 30 mg per kilo, were given intravenously there was no manifestation of symptoms. A dose not over 50 mg per kilo may, therefore, be regarded as the minimum toxic dose when injected intravenously under the conditions stated.

SERIES D.

A 0.5 per cent caffein solution was used in these experiments in order to test the effect of concentration on its toxicity; the rate of injection was 1 cc per minute.

Rabbit 569. Gray male. Weight, 1,475 grams. Diet, oats.

February 6: 11.50 a. m. to 12.01 p. m., injected 10 cc 0.5 per cent caffein; 12.03 to 12.12 p. m., injected 10 cc of 0.5 per cent caffein; 12.13 to 12.26 p. m., injected

10 cc of 0.5 per cent caffein, total amount injected, 30 cc; 12.20, passed 35 cc of urine; 12.30, increased reflexes, but no convulsions; 4 p. m., reflexes increased.

February 11: Alive, condition good.

Rabbit 574. Gray female. Weight, 1,555 grams. Diet, oats.

February 8: 10.25 to 10.33 a. m., injected 4 cc of 0.5 per cent caffein in salt solution, injection discontinued for five minutes; 10.38 to 11.10, injected 30 cc, total amount of caffein solution received, 34 cc; 11.55 a. m., very sensitive; reflexes markedly increased.

February 9: Alive, condition good.

Rabbit 571. Gray female. Weight, 1,530 grams. Diet, oats.

February 7: Injection 3.18 to 3.50 p. m., received 30 cc in 32 minutes, not hypersensitive; 3.55, restlessness and weakness of extremities; 4.10 p. m., control of anterior extremities impaired, distinctly paretic but tried to walk about, died the same afternoon.

Rabbit 568. Gray male. Weight, 1,605 grams. Diet, oats.

February 7: Injection 10.53 to 11.01 a. m., injected 10 cc 0.5 per cent caffein; 11.03, injection resumed after two minutes interval; 11.14, received 10 cc 0.5 per cent caffein intravenously in 11 minutes; 11.16, injection resumed; 11.35, received 12 cc 0.5 per cent caffein, total amount of caffein solution received, 32 cc; 12.30 p. m., urinated 14 cc of bloody urine; 12.55 p. m., convulsions and death a few minutes later. Autopsy showed congestion of viscera, but no other lesions.

Rabbit 570. Gray female. Weight, 1,225 grams. Diet, oats.

February 7: 2.06 to 2.35 p. m., injected 24.5 cc 0.5 per cent caffein, reflexes increased but no convulsions, paresis especially marked in the anterior extremities; 3 p. m., passed urine which was normal in appearance, reflexes not increased but rabbit was weak.

February 9: Found dead. *Autopsy:* Liver, spleen, and kidneys congested; large intestines hemorrhagic; omentum congested and showed the presence of small caseous nodules; liver showed adhesion to diaphragm; viscera presented the appearance of intraabdominal infection.

Of the five rabbits of this series three died as a result of the administration of caffein. The other two which survived showed mild symptoms only, such as increased reflexes, but no evidence of severe poisoning such as was observed after the injection of the same doses of caffein in series A when a 2 per cent solution of caffein was injected. Convulsions were noticed in one case only (No. 568); paresis in two cases (Nos. 570 and 571). The nervous symptoms even in this group, therefore, were much milder than in series A. The percentage of death, however, was greater than in series A, in which the concentration of caffein was four times as great. It is quite probable that the strain on the heart due to the sudden increase in volume of the blood and its dilution might be an important factor in increasing the toxicity of caffein. It is conceivable that doses just sufficiently large to depress the normal heart may cause paralysis of an already overstrained organ.

SERIES E.

In the two experiments of this series the rate of injection as a possible factor influencing the toxicity of caffein was tested. A 2 per cent caffein solution was injected at the rate of 1 cc in two and one-half to three minutes.

Rabbit 572. Gray male. Weight, 1,770 grams. Diet, oats.

February 8: Injection began at 3 p. m., discontinued at 3.37 p. m., and resumed at 3.38 p. m.; rabbit was restless; injection finished at 3.52 p. m. Total quantity received, 17.4 cc 2 per cent caffein intravenously in 52 minutes; struggled intermittently during the injection; anterior legs paralyzed.

February 9: Found dead.

Rabbit 573. Gray male. Weight, 1,810 grams. Diet, oats.

February 8: Started to inject at 1.35 and discontinued at 2.27 p. m.; received 18 cc 2 per cent caffein intravenously in 52 minutes; reflexes markedly increased soon after; 2.45, passed bloody urine; 4.30 p. m. reflexes increased; no other symptoms.

February 9: 9 a. m., found dead.

It will be observed that some retardation of the onset of symptoms was caused by slower injection, but the final result was the same as when the injections were made more rapidly. It is quite probable, therefore, that a much slower rate of injection may lessen considerably the toxicity of caffein.

From the results of the experiments by intravenous injection summarized in the table, it appears that the minimum toxic dose for rabbits of a 2 per cent caffein solution, injected at the rate of 1 cc per minute, is about 50 mg per kilo. Twice the dose induces severe symptoms and may be fatal; 160 mg per kilo are surely fatal. If the rate of injection is diminished, the toxicity of caffein is lessened, but this effect is not marked unless the injections are very slow. Dilution of the caffein solution suppresses to some extent the nervous symptoms, but the toxicity, on the contrary, seems to be increased.

TABLE 5.—*Intravenous injections.*

No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.	Diet.	Remarks.
				SERIES A.		
	Grams.	*Mg.*				
194	1,310	114	Present...	Survived....	Oats......	White female.
556	1,635	134	10 minutes	20 minutes....	...do......	Gray female.
557	1,580	114	Present...	Survived....	...do......	Do.
558	1,590	100	...do......	.....do....	...do......	Do.
292	1,770	141	...do......	.....do....	...do......	Do.
194	1,350	126	...do......	10 minutes....	Carrots....	Do.
				SERIES B, GROUP I.		
562	1,650	200	....	1⅓ hours....	Oats......	Gray female.
561	1,450	200	....	.....do....	...do......	Do.
560	1,620	200	Present...	Less than 24 hours....	...do......	Do.
559	1,875	190	...do......	Survived....	...do......	Do.
				SERIES B, GROUP II.		
279	1,320	166	....	1 hour....	....	Gray and white female.
254	1,285	160	....	Survived....	Oats......	Gray female.
567	....	162	....	About 45 minutes....	....	Do.
255	....	158	....	Died....	....	
				SERIES C.		
293	1,610	500	Present...	Survived....	Oats......	Gray female.
227	2,320	570	None......	.....do....	....	White male.
563	1,650	500	Present...	.....do....	...do......	Gray female.
564	1,515	460	...do......	.....do....	....	Do.
565	1,545	320	None......	.....do....	...do......	Do.
566	1,900	310	...do......	.....do....	...do......	Do.
				SERIES D.		
569	1,475	100	Present...	Survived....	Oats......	Gray male.
574	1,555	112	...do......	.....do....	...do......	Gray female.
571	1,530	100	...do......	About 2 hours....	...do......	Do.
568	1,605	100	....	20 minutes....	...do......	Gray male.
570	1,225	100	....	Less than 20 hours....	...do......	Do.
				SERIES E.		
572	1,770	200	Present...	About 24 hours....	Oats......	
573	1,810	200	....	.......do....	...do......	

SUMMARY.

The results of the experiments on rabbits show considerable variation in the toxicity of the single dose. Individuals differed so widely in their resistance to this drug that the same experiments had to be repeated many times with each method of administration before satisfactory conclusions could be drawn. This is strikingly illustrated in the experiments by intravenous injection in which a dose of nearly 0.2 gram per kilo was not fatal. Similar instances of exceptional resistance or of sensitiveness to caffein were observed when it was given in other ways. A comparison of the toxicity of caffein administered by different methods in this investigation shows well-marked differences in its activity, although they are not quite so striking as similar experiments with other alkaloids reported by several observers. The toxicity of caffein in these experiments on the rabbit indicates that it is greatest when given by vein and least when given by mouth. The ratio of the minimum toxic doses by these two methods of introduction of caffein was about 7.1; the relation of the minimum fatal dose was about 3.1. The toxicity when given subcutaneously is about 15 to 20 per cent greater than when given by mouth. The difference between the intramuscular and subcutaneous injection is even more marked. The toxicity of caffein when injected into the muscles is about midway between that administered by the subcutaneous and intraperitoneal routes, and is about half that injected intravenously. Meltzer and Auer,[58] who experimented with a number of drugs found that the intramuscular method of administration is as effective as the intravenous, fluorescin forming the only exception according to their observations. In the experiments of Sollman and Brown[51] with ergot, the effect was quite different from those obtained by Meltzer and Auer[58] with the drugs they used. It is quite possible that the result obtained with ergot is merely illustrative of a difference in the behavior of various substances in this regard. This appears probable on account of the difference in the rate of absorption for various substances. Thus, according to Achard, Gaillard, and Ribot (Compt. rend. Soc. biol., 1907, *62*: 90), absorption from the peritoneal cavity varies with the concentration of the solution and the size of the molecule. The smaller the molecule and the greater the concentration the more rapid the absorption. That the rate of absorption from the intramuscular tissues is unequal and varies for different substances appears from the experiments of Meltzer and Auer.[58] The difference was very striking between intramuscular and subcutaneous administration of curara or adrenalin; the results were somewhat different with morphin and with fluorescin. As shown in their protocols, the onset of the symptoms after the intramuscular injection of morphin was sooner than after subcutaneous injection, but in time the difference diminishes and disappears altogether. The absorption of fluorescin is much faster when the intramuscular path is used than when given subcutaneously, but the writers state that the rate falls far behind that of the intravenous administration. The difference in toxicity we observed between feeding by mouth and subcutaneous injection, although distinct, was not very great. It was much less than Maurel[55] obtained with the hydrobromid of caffein in the rabbit. Whether this difference between his results and ours is due to the use of the pure alkaloid in our experiments and the hydrobromid employed by Maurel can not be stated at present with any degree of accuracy. It is hoped that the work in progress in the laboratory will throw some light on the subject in the near future. But Maurel's[55] experiments show that various substances behave differently in this regard. Thus the toxicity of strychnin, he states, is three times as great when given subcutaneously as when given by mouth and six times that of the minimum fatal dose by vein. It may be remarked, however, that examination of his data shows that his doses are much too large for the rabbit. In experiments with other drugs little or no difference between the two modes of administration was noticed. Thus, digitalin was but slightly more active when given subcutaneously than by mouth, while the toxicity of emetin hydrochlorid was just the same, whichever one of these methods of introducing the substance was used. Differences in the toxicity of substances have also been observed between subcutaneous and intravenous modes of administration, but here, too, the differences for various substances were unequal.

EXPERIMENTS ON GUINEA PIGS.

The toxicity of caffein was studied in a large number of individuals. The experiments were conducted on full-grown animals and were carried out at different seasons of the year in a variety of ways. Special attention was given to diet as a possible factor influencing resistance to caffein, and the effect of different modes of administration on toxicity. Some animals were therefore fed oats, some carrots, others received both hay and oats. Caffein was introduced subcutaneously, intraperitoneally, and by mouth.

SUBCUTANEOUS INJECTION.

SERIES A.

Preliminary experiments carried out on three guinea pigs, which received 360, 300, and 290 mg of caffein per kilo subcutaneously have shown that such doses were rapidly fatal. Two of the animals were seized with convulsions half an hour after the introduction of caffein and died during the attack. The other had tetanus two minutes after the injection of caffein. Repeated attacks followed, which terminated in the death of the animal two and a half hours later. The fatal and toxic doses must therefore be considerably under 0.3 gram of caffein per kilo when introduced by this path and smaller doses were therefore injected. The results are shown in the experiments of the next series.

SERIES B.

Experiments with 2 decigrams per kilo constituted this series.

Guinea pig 20. Female. Weight, 497 grams. Diet, oats.

April 2: 5 cc 2 per cent caffein injected subcutaneously at 11.30 a. m.; 1.50 p. m., spasm of short duration. Died at 3 p. m., three and one-half hours after injection.

Guinea pig 38. Brown male. Weight, 570 grams. Diet, carrots and oats week previous to injection.

February 11: 3.50 p. m., 6 cc 2 per cent caffein injected subcutaneously in back (210 mg per kilo); 4.15, reflexes increased, had convulsion of short duration when disturbed; 4.45 p. m., on handling, repeated convulsion and paralysis; 5 p. m., guinea pig lying on his side, respiration difficult and labored.

February 11: 5.05 p. m., guinea pig found dead, 2 hours and 15 minutes after injection.

Guinea pig 37. Male. Weight, 820 grams. Diet, carrots and oats during week preceding the injection of caffein.

February 11: 3.35 p. m., 8.5 cc 2 per cent caffein injected subcutaneously in the back; 5 p. m., pig very sensitive, anterior extremities paralyzed when handled, frequent spasms of posterior extremities, no symptoms noticed before 5 p. m., although watched all the time; 5.05 p. m., guinea pig on his legs and looked normal. No attack on handling.

February 12: 9 a. m., found dead; died within 18 hours.

Guinea pig 13. Female. Weight, 618 grams. Diet, oats.

March 29: 2.45, 6 cc 2 per cent caffein injected subcutaneously (0.194 grams per kilo).

March 30: Died at 4 p. m., 25 hours after injection.

Guinea pig 36. Male. Weight, 850 grams. Fed oats and carrots for one week previous to injection.

February 11: 3.30 p. m., 8.5 cc 2 per cent caffein injected subcutaneously into back; 5 p. m., somewhat more sensitive than normal, no other symptoms, no effect on handling; 5.05 p. m., no symptoms.

February 12: 9 a. m., found dead, about 18 hours after injection.

The results of these experiments, as observed in five guinea pigs, indicate that two decigrams of caffein per kilo of animal produce symptoms within a half to about two and a quarter hours after injection. Death followed in two guinea pigs 70 minutes to 1 hour after the first manifestations of symptoms. Two others died during the night, while one lived 25 hours after the injection of caffein. Even 2 decigrams caffein per kilo weight might therefore be fatal to the guinea pig. Experiments carried out later have shown, however, that the resistance to caffein is appreciably greater in some guinea pigs. This is indicated by the following experiments, in which doses of 0.2 to 0.24 gram caffein per kilo were administered by the same path.

SERIES C.

Guinea pig 66. Yellow and dark brown male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., alive; condition good.

October 9: Found dead. *Autopsy:* Congestion of liver, kidney, and small intestine.

Guinea pig 65. White and black male. Weight, 510 grams. Diet, oats.

October 4: 5 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously in the back at 3 p. m.; 5 p. m., no symptoms.

October 5: 9 a. m., condition good.

Guinea pig 60. White and gray female. Weight, 320 grams. Diet, oats.

October 3: 2.25 p. m., 3.5 cc 2 per cent caffein ((0.219 gram per kilo) injected subcutaneously in the back; 3.40 p. m., convulsion with recovery; 3.50 p. m., frequent spasms with paralysis, especially of anterior extremities; 5.30 p. m., tetanus when removed from cage and put on floor.

October 4: 8.50 a. m., found dead. *Autopsy:* Congestion of small intestines, lungs, liver.

Guinea pig 57. White and gray female. Weight, 350 grams. Diet, oats.

October 3: 2.15 p. m., 3.5 cc 2 per cent caffein injected subcutaneously in the back (0.2 gram per kilo); 3.40 p. m., convulsions with recovery; 5.30 p. m., no marked symptoms.

October 4: 8.50 a. m., alive, active.

October 6: Found dead at 9 a. m. *Autopsy:* Congestion of lungs and liver; kidneys petechiated; severe gastro-enteritis.

Guinea pig 68. Yellow male. Weight, 785 grams. Diet, oats.

October 6: 11.35 a. m., 7.8 cc 2 per cent caffein (0.2 gram per kilo) injected subcutaneously; 12 noon, reflexes increased markedly; 4.20 p. m., reflexes the same as at 12 noon.

October 7: 9 a. m., dead. *Autopsy:* Lungs congested; liver congested and fatty; spleen congested, kidney showed hemorrhagic spots; gastric mucosa necrotic; small portion of small intestine inflamed.

Guinea pig 69. White male. Weight, 585 grams. Diet, oats.

October 6: 11.40 a. m., 5.8 cc 2 per cent caffein injected subcutaneously; 12 noon, reflexes increased, but not as much as in No. 68; 4.20 p. m., guinea pig hypersensitive, reflexes increased more than at 12 noon.

October 7: 9 a. m., alive.

October 15: 9 a. m., found dead.

Guinea pig 61. Brown and black female. Weight, 330 grams. Diet, oats.

October 3: 4 p. m., 4 cc 2 per cent caffein (240 mg per kilo) injected subcutaneously; 5.30 p. m., reflexes increased; runs, but drags posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 62. White, yellow, and black female. Weight, 335 grams. Diet, oats.

October 3: 4.05 p. m., 4 cc 2 per cent caffein (238 mg per kilo) injected subcutaneously in the back; 5 p. m., convulsions; 5.20 p. m., convulsions, alternating with paralysis of anterior and posterior extremities.

October 4: 8.50 a. m., found dead.

Guinea pig 70. White and brown male. Weight, 545 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (238 mg per kilo) aqueous solution injected subcutaneously; 3.50 p. m., reflexes increased.

October 9: 9 a. m., found dead.

Guinea pig 71. Brown and white male. Weight, 540 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein solution (0.24 gram per kilo) injected subcutaneously; 3.45 p. m., reflexes increased, tetanus.

October 9: 9 a. m., found dead.

Guinea pig 72. Brown and white male. Weight, 560 grams. Diet, oats.

October 7: 3 p. m., 6.5 cc 2 per cent caffein (0.232 gram per kilo) aqueous solution administered by subcutaneous injection; 3.35 p. m., reflexes increased.

October 10: found dead. *Autopsy:* Nos. 70, 71, 72 showed congestion of organs.

The reaction to caffein in the experiments of this series (C) showed considerable variation. The appearance of symptoms, as well as the final outcome of the experiments, differed markedly in a number of cases, notwithstanding the fact that the conditions were the same; thus the administration of 0.2 gram per kilo to guinea pigs, all of which received the same diet, induced no symptoms in two of the animals (Nos. 66 and 65), while marked symptoms were observed in the other four; in two of these the symptoms appeared in one hour and a quarter after injection, and in two others (Nos. 68 and 69), mild symptoms only appeared in 20 or 25 minutes. The last two were under observation for 4 hours longer, but there was no visible change in their condition. The duration of life in all of these guinea pigs, as indicated in the table, likewise varied. Two (Nos. 60 and 68) died during the night after they received caffein, one survived (No. 65), and three others (Nos. 57, 66, and 69) lived 2¼, 5, and 9 days, respectively. Experiments with larger doses likewise showed differences in the behavior of these animals toward caffein, but they were not quite so marked. As shown in the table, symptoms appeared in from 35 minutes to 1.5 hours after injection. The duration of life was less than 1 day in two pigs, about twice as long in two others, and in one case between 2 and 3 days.

A comparison made with results obtained in the preceding series shows a striking difference in the resistance to caffein. As 2 decigrams per kilo proved more rapidly fatal to the guinea pig than the larger doses employed in the later experiments, this difference in the resistance to caffein may be due to several factors. As pointed out in the experiments on rabbits, age might be an important factor influencing the toxicity of caffein. Unfortunately, no accurate data were available on the age of the guinea pigs, but they were all apparently full grown, although they differed in weight considerably. The difference in their ages was in all probability not very great. Moreover, it will be observed that the resistance in series B and C differed in animals of approximately the same weight. This is evident on comparing experiments Nos. 20, 38, and 13 of series B with Nos. 65, 66, and 69 of the next series. Again, further inspection and analysis of these tables show no difference in the toxicity, although there may be considerable difference in the weight, from which it may be concluded that the animals were of about the same age or that this plays no part in the resistance to caffein in the guinea pig.

Diet is another factor which should be taken into consideration in this connection. The recent work of Hunt[39] indicates that this may influence the resistance of animals to some poisons. Our experiments, however, fail to show any difference in the toxicity of the caffein in guinea pigs, whether fed oats, carrots, or both, for different results were obtained on the same diet, and there seemed to be little or no difference in the toxicity of caffein when the diet was different. Other explanations suggest themselves to account for the results obtained. Seasonal changes have been assigned by a number of investigators as a cause of variation in the resistance to drugs. According to Focke,[24] frogs are more susceptible to digitalis in the spring than in the summer, while Moschkowitsch [61] and Edmunds [21] reported the very opposite results. Schmiedeberg's [80] observations on strophantin in frogs were in harmony with those of Edmunds [21] and Moschkowitsch.[61] Similar results were reported with guinea pigs. Harrington's [34] experiments indicate that stimulation of the vagus is less effective from October to January than from February to April, when they are also much more susceptible to operative procedure. Hunt found that the resistance of guinea pigs to aceto nitril is about twice as great in the summer months as it is in January and February.

Race might also be thought of as an important factor in this connection. Since the guinea pigs used at different seasons of the year were of several varieties, there is no reason to suppose, however, that the varieties experimented upon in the summer were more resistant than those used in the winter and spring. It is highly probable, therefore, that the greater resistance to caffein of the guinea pigs of series C than those of series B was due to seasonal variation.

Doses of 0.20 to 0.24 gram caffein per kilo weight, therefore, may be regarded as the minimum fatal dose for the guinea pig, depending upon the season. Since 0.2 gram per kilo proved to be rapidly fatal in series B, this quantity was perhaps not the minimum fatal dose for the guinea pig at the season during which the experiments were made. Additional tests with smaller doses were therefore carried out during February and March. The results are shown in series D.

SERIES D.

Guinea pig 49. Male. Weight, 510 grams. Diet, oats for 1 month previous to experiment.

March 17: 3 p. m., 4 cc 2 per cent caffein (0.16 gram per kilo) were injected subcutaneously; 4.40 p. m., reflexes increased; 5.40 p. m., no symptoms.

March 18: 9 a. m., found dead, died in less than 18 hours. *Autopsy:* Hemorrhage into abdominal cavity; liver and spleen unduly congested; intestines injected; hemorrhagic area at point of injection.

Guinea pig 40. Male. Weight, 630 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc 2 per cent caffein (0.158 gram per kilo) injected subcutaneously into back.

February 13: 1 p. m., still alive.

February 14: 9 a. m., found dead.

Guinea pig 45. Female. Weight, 435 grams. Diet, oats for about one month previous to injection.

March 17: 3 p. m., 3.5 cc of 2 per cent caffein injected subcutaneously in the back (0.160 gram per kilo); 4.35 p. m., no symptoms; 5.40 p m., no symptoms.

Guinea pig 39. Male. Weight, 820 grams. Diet, oats and carrots.

February 12: 11 a. m., 6 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously in back.

February 14: 9 a. m., alive; seemed to be in good condition; found dead at 1 p. m.

Guinea pig 41. Weight, 660 grams. Diet, oats and carrots one week previous to injection.

February 12: 11 a. m., 5 cc (0.15 gram per kilo) 2 per cent caffein injected subcutaneously.

February 14: 2 p. m., pig alive; apparently normal.

February 18: Guinea pig still alive and apparently in good condition.

Guinea pig 46. Female. Weight, 470 grams. Diet, oats about one month previous to experiment.

March 17: 3.15 p. m., 4 cc (0.170 gram per kilo) 2 per cent caffein injected into back subcutaneously; 4.35 p. m., reflexes increased, tremors on handling marked; 5.40 p. m., no change, symptoms about as before.

March 18: 2.30 p. m., no symptoms.

The experiments of this series (D) likewise showed a considerable difference in the resistance of the individual guinea pigs. Nos. 41, 45, and 46 survived; the rest of the pigs died within 18 hours to 2 days after the administration of caffein. Since an autopsy was held on one only, it is impossible to assign a cause for the variation in the toxicity of caffein in these guinea pigs, as the diet and the other conditions under which the experiments were conducted were the same. It was found in the experiments on cats and rabbits that the presence of morbid processes tends to increase the toxicity of caffein. The observations of Ophüls[66] are of interest in this connection. He found spontaneous lesions of the kidney and liver in a large proportion of guinea pigs examined. The greater susceptibility to caffein of guinea pigs Nos. 39, 40, 49, is probably due therefore to some pathological change which increased its toxicity. About 0.2 to 0.24 gram per kilo may therefore be regarded as the minimum lethal dose for the normal guinea pig when caffein is introduced subcutaneously, the minimum toxic dose being about 150–160 mg per kilo.

Experiments were also conducted to determine the largest dose which does not pro-uce any visible effects. In a number of tests with from 100 to 120 mg caffein per ilo (series E, see Table 6, p. 51) no manifestation of nervous or muscular disturbance or any departure from the normal in respiratory activity was observed. Such uantities may be regarded as the largest doses which are surely safe for these animals. t is quite possible, therefore, that the greater variation in the toxicity of caffein bserved in these experiments is due to morbid conditions. Moreover, there is some vidence that caffein increases the toxicity of certain poisons, as shown by Hale[33] for cetanilid. Is it not possible that caffein may similarly be affected by poisons cir-ulating within the body? Indeed the recent work of Loeb[28] makes this supposition ighly probable. This investigator found that caffein and adrenalin injected together roduce myocarditis in the rabbit. It is conceivable that the combined action of affein and some preexisting poison may cause changes which terminate in the death f the animal. The delayed death of guinea pigs after the administration of caffein bserved in this and other series may probably be accounted for in this way.

Experiment 57 lends some support to this view. The condition of the kidneys and he presence of a severe gastro-enteritis are sufficient to account for the death of this ase. Again the frequent association of gastro-enteritis and congestion of the organs n caffein intoxication found in different animals makes it highly probable that these lesions were caused by caffein.

INJECTION INTO THE PERITONEAL CAVITY.

The experiments were carried out with different doses. All the guinea pigs in this series were kept on a uniform diet, consisting of oats. Most of them were of average size and there were no wide variations in their weights. The experiments of series A with the smallest doses were conducted in March and April; all the other experiments it will be noticed were made in October.

SERIES A.

Guinea pig 41. Weight, 700 grams. Diet, oats.

April 1: 3.30 p. m., 4.5 cc 2 per cent caffein (130 mg per kilo) injected into peritoneal cavity. 5.35 p. m., symptoms present but no tetanus.

April 2: Found dead about 2 p. m., duration of life about 22 hours. *Autopsy:* Subcutaneous hemorrhage at the point of inoculation; serious exudate on visceral and parietal peritoneum with marked inflammation of peritoneum; portions of intestines showed slight enteritis.

Guinea pig 49. Male. Weight, 370 grams. Diet, oats.

April 1: 3.15 p. m., 2.5 cc 2 per cent caffein (135 mg per kilo) injected into the peritoneal cavity; 5.30 p. m., symptoms present; reflexes increased, but no tetanus. Guinea pig survived.

Guinea pig 47. Female. Weight, 550 grams. Diet, oats since about February 4.

March 17: 3.30 p. m., 3.5 cc 2 per cent caffein (127 mg per kilo) injected into peritoneal cavity; 4.35 p. m., increased irritability present, but not marked; 5.40 p. m., symptoms about the same as before.

March 18: 2.30 p. m., condition good; no symptoms. Survived.

Guinea pig 50. Female. Weight, 290 grams. Diet, oats.

April 1: 3.30 p. m., 2 cc 2 per cent caffein (138 mg per kilo) injected into peritoneal cavity; 5.35 p. m., symptoms present; reflexes much increased, but no tetanus. Survived.

SERIES B.

Guinea pig 51. Yellow female. Weight, 415 grams.

October 1: 9.50 a. m., 3 cc (144 mg per kilo) 2 per cent caffein injected into peritoneal cavity; 4.30 p. m., no symptoms, although under observation all day.

October 3: 2 p. m., alive.

Guinea pig 52. White male. Weight, 450 grams.

October 1: 9.45 a. m., 3.5 cc, 2 per cent caffein (155 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

Guinea pig 53. Brown and white male. Weight, 490 grams.

October 1: 9.45 a. m., 4 cc, 2 per cent caffein (163 mg per kilo), injected into peritoneal cavity; 4.30 p. m., no symptoms developed since injection.

October 3: 2 p. m., alive.

October 8: Found dead. *Autopsy:* Congestion of lungs, spleen, liver, kidneys, and small intestines.

SERIES C.

Guinea pig 59. Gray and white. Weight, 375 grams. Diet, oats.

October 3: 2 p. m., 3.75 cc (0.2 gram per kilo) injected into peritoneal cavity; 2.15 p. m., reflexes increased but not markedly; 4 p. m., reflexes still more increased; no other symptoms; 5.30 p. m., no symptoms.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 58. Brown and white. Weight, 380 grams. Diet, oats.

October 3: 2 p. m., 3.8 cc caffein (0.2 gram per kilo), 2 per cent solution, injected into peritoneal cavity; 2.10 p. m., hind legs extended, then tetanus; attack lasted a few seconds, after which pig raised himself on his legs, but reflexes remained much exaggerated; 4 p. m. to 5.30 p. m., no symptoms of caffein intoxication.

October 4: 8.50 a. m., guinea pig alive and active.

Guinea pig 56. Gray and white male. Weight, 440 grams. Diet, oats.

October 1: 11.30 a. m., received 4.6 cc of 2 per cent caffein solution (0.2 gram per kilo) into abdominal cavity; 11.45 a. m., stiffness and rigidity of posterior extremities, reflexes increased; 12.30 p. m., hind legs paralyzed, reflexes increased; 4.35 p. m., no symptoms, guinea pig in good condition.

October 3: Still alive, in good condition.

October 14: Died. *Autopsy:* Anterior lobe of right lung hepatized. Small portion of small intestine edematous. Other organs normal.

Guinea pig 55. White and yellow male. Weight, 690 grams. Diet, oats.

October 1: 11.30 a. m., received 6.5 cc of 2 per cent solution caffein (188 mg per kilo) into peritoneal cavity; 11.40 a. m., stiffness in all extremities, reflexes markedly increased; 12.30 p. m., reflexes increased, anterior and posterior extremities paralyzed; 3 p. m., found dead.

SERIES D.

Guinea pig 67. Gray and yellow male. Weight, 330 grams. Diet, oats.

October 5: 11.25 a. m., 4 cc of 2 per cent caffein injected into peritoneal cavity (240 mg per kilo); 11.30 a. m., tetanus—survived, convulsions off and on. Death at 2.55 p. m. *Autopsy:* Severe gastroenteritis; kidney petechiated; congestion of lungs and liver.

Guinea pig 63. Gray and white male. Weight, 340 grams. Diet, oats.

October 5: 11.20 a. m., 4 cc of 2 per cent caffein (235 mg per kilo) injected into peritoneal cavity.

October 14: Alive and in good condition.

Guinea pig 64. Brown and black female. Weight, 305 grams.

October 5: 11.35 a. m., 3.8 cc 2 per cent solution caffein (250 mg per kilo) injected into peritoneal cavity; 11.40 a. m., tetanus—survived, convulsions off and on, died at 4.15 p. m. *Autopsy:* Findings exactly the same as in No. 67.

Examination of the results of the experiments by intraperitoneal injections showed that 0.2 gram caffein per kilo was toxic in two guinea pigs (Nos. 59 and 58). Severe symptoms were observed within 15 minutes in No. 56 and within one hour in No. 55 after the administration of approximately the same dose of caffein. One of these died within three and one-half hours; the other, No. 56, made a good recovery from the acute effects. This amount of caffein may be regarded, therefore, as the minimum toxic dose for the guinea pig when injected into the peritoneal cavity. This is corroborated by the experiments of series B in which smaller doses failed to show any muscular, nervous, or respiratory symptoms, nor were there any after effects noticed, as all of them survived and were kept under observation for some time. The guinea pigs of series A, however, seem to contradict these results. It will be remarked that appreciably smaller doses induced symptoms in all of them, and one case terminated fatally. The seasonal variation, as already pointed out, is in all probability likewise responsible for the difference in the resistance between the guinea pigs of series A and B. Tests were made also to determine the minimum fatal dose. For this purpose the experiments of series D were performed. The resistance of No. 63 in this series is quite striking. We are unable to explain such a discrepancy in the results obtained under practically uniform conditions. The minimum fatal dose of caffein, when injected into the peritoneal cavity, is therefore about 240 to 250 milligrams per kilo. These amounts, it will be observed, were rapidly fatal, in striking contrast to the results obtained when such doses were injected subcutaneously. This is probably due to a better absorption from the peritoneal cavity than from the subcutaneous tissues.

ADMINISTRATION BY MOUTH.

All the guinea pigs in these experiments were kept on a diet of hay and oats and were of large size. The tests were made with different doses of caffein in order to determine the limits of toxicity when the drug was administered by mouth.

Guinea pig 129. White and black male. Weight, 855 grams. Diet, oats and hay.

June 6: 2.20 p. m., 12 cc of 2 per cent caffein (0.28 gram per kilo) by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more increased; no other symptoms.

June 7: 9 a. m., found dead; guinea pig passed 75 cc urine, which was almost colorless. *Autopsy:* Heart and blood vessels injected; lungs congested; small intestines congested; other organs apparently normal.

Guinea pig 130. Black and brown male. Weight, 800 grams. Diet, oats and hay.

June 6: 2.30 p. m., 12 cc of 2 per cent caffein (0.3 gram per kilo) administered by mouth; 3 p. m., reflexes increased; 5 p. m., increase of reflexes greater than at 3 p. m.

June 7: 9 a. m., found dead; only a few cubic centimeters of urine passed since 4 p. m. *Autopsy:* Heart and blood vessels injected; lungs congested; small intestines congested slightly.

Guinea pig 131. White and yellow male. Weight, 860 grams. Diet, oats and hay.

June 6: 2.40 p. m., 12 cc 2 per cent caffein administered by mouth; 3 p. m., reflexes increased; 5 p. m., reflexes still more marked.

June 7: 9 a. m., found dead, pig passed about 5 cc urine since 4 p. m. of previous day. *Autopsy:* Same as in No. 130.

Guinea pig 136. White and black male. Weight, 1,000 grams. Diet, oats and hay.

June 9: 4 p. m., 7.5 cc 2 per cent caffein solution injected subcutaneously into the back; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., more sensitive than normal guinea pigs, but reflexes not quite so marked as at 5 p. m. previous day, about 15 cc urine passed since caffein was injected, reduction of Fehling's solution considerable, no albumin.

June 13: Alive and in good condition. Appetite good. (NOTE.—Parallel test with urine from two guinea pigs which did not receive caffein failed to show reduction of Fehling's solution.)

Guinea pig 137. White and brown male. Weight, 925 grams. Diet, oats and hay.

June 9: 4 p. m., 7 cc 2 per cent solution caffein injected subcutaneously; 4.50 p. m., reflexes increased.

June 10: Reflexes less marked than at 5 p. m. previous day, but is more sensitive than normal guinea pig, about 10 cc urine passed since injection of caffein, moderate amount of reduction of Fehling's solution.

June 13: Guinea pig alive, appetite good, condition good.

June 16: 9 a. m., found dead.

Guinea pig 135. White and black male. Weight, 955 grams. Diet, hay and oats.

June 9: 3 p. m., 7.5 cc 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: Reflexes less than on previous day and less marked than in No. 136, a few cubic centimeters dirty brown urine collected but could not be tested for reduction.

June 13: Condition good, appetite good.

June 16: 9 a. m., found dead.

Guinea pig 134. White and brown male. Weight, 740 grams. Diet, hay and oats.

June 9: 2.55 p. m., 6 cc warm 2 per cent caffein solution given by mouth through stomach tube; 4.50 p. m., reflexes increased.

June 10: 9.30 a. m., reflexes much less than day before, increase slight, a few cubic centimeters of urine passed since injection of caffein, looked brown and dirty, could not be tested for reducing substances.

June 13: Guinea pig alive, appetite good, condition good.

June 14: 9 a. m., found dead.

Guinea pig 128. White and black male. Weight, 1,075 grams. Diet, hay and oats.

June 7: 10 a. m., 11 cc 2 per cent caffein by mouth through stomach tube; 11.10 a. m., no symptoms, no urine passed; 1 p. m., increased reflexes, about 15 cc (estimated) urine passed; 4 p. m., reflexes increased, still more urine passed (about 20 cc); 4.50 p. m., tetanus, frequent attacks, then paralysis and death at 4.58 p. m. *Autopsy:* Lungs congested; blood vessels of heart injected; intestines slightly congested; fatty liver.

Guinea pig 126. White and gray male. Weight, 980 grams. Diet, oats and hay.

June 7: 9.40 a. m., 9.8 cc 2 per cent caffein given by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., no urine passed, reflexes increased; 1 p. m., more sensitive than before; 4 p. m., increase of reflexes more marked, no urine passed; 4.45 p. m., about 15 cc urine collected; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day, no urine passed since 4.45 p. m. previous day, considerable reduction of Fehling's solution, much more than urine of guinea pig No. 127; 11.05 a. m., convulsions; 12 noon, still alive and stretched out on abdomen; died at 1 p. m. *Autopsy:* Lungs badly congested; heart and blood vessels injected; blood vessels of kidney and of small intestines injected; liver engorged with blood; a few necrotic spots in stomach.

Guinea pig 127. White, black, and brown male. Weight, 760 grams. Diet, oats and hay.

June 7: 9.50 a. m., 7.6 cc 2 per cent caffein by mouth through stomach tube; 10 a. m., no symptoms; 11.10 a. m., reflexes increased, no urine passed; 1 p. m., very sensitive; 4 p. m., sensitiveness increased, about 20 cc urine passed; 5 p. m., no change.

June 8: 9 a. m., reflexes about the same as 5 p. m. previous day; 9.30 a. m., guinea pig passed 30 cc urine since he received caffein, urine showed a moderate amount of reduction; 12 noon, convulsions; died at 2.30 p. m. *Autopsy:* Lungs congested; blood vessels of heart and of intestines injected; numerous necrotic spots in stomach; other organs apparently normal.

Examination of the protocols shows that the absorption of caffein from the gastro-intestinal canal was quite rapid, symptoms having been observed as early as 20 minutes after its introduction. The duration of life, it will be remarked, varied with the size of the dose. When approximately 3 decigrams per kilo were fed, all the animals died in the night. They lived, therefore, less than 18 hours. Two decigrams per kilo were likewise fatal, but the duration of life was longer. To decide whether or not this is the smallest fatal dose, smaller amounts were fed. It seemed at first that about 150 mg per kilo was the smallest toxic dose, and about 200 mg per kilo the minimum fatal dose. Macroscopic examination of the organs, however, threw some doubt on this supposition, for well-marked lesions were noticed in all of the guinea pigs which received 0.2 gram per kilo. It is quite possible, therefore, that the minimum fatal dose may be somewhat higher, as we have reason to believe that, at least in some pathologic conditions, the susceptibility to caffein is increased. The presence of fatty changes in the liver of No. 128 and the rapid death in this case lends especial support to this view. Hence, the minimum fatal dose is probably greater than 0.2 gram per kilo for the normal guinea pig. The doses employed for the tests on guinea pigs Nos. 129, 130, and 131 may be considered therefore the minimum fatal dose for these animals. It will be also remarked that macroscopical examination of the organs of these animals failed to reveal the presence of severe lesions. That the minimum toxic dose is probably much smaller than 0.28 gram per kilo is indicated by the experiments on guinea pigs Nos. 135 and 134, in which 0.15 gram caffein per kilo induced mild symptoms in from two to three hours. Both of these, however, and also No. 137 died four to six days after the drug was fed. As already pointed out, caffein may be a factor in the delayed death of guinea pigs which received moderate doses of it. That this supposition may also be true for guinea pigs Nos. 134, 135, and 137 is indeed made probable by the observation that after moderate amounts of caffein symptoms may persist in the guinea pig for about 24 hours, and also by the fact that the secretion of urine in these animals was very scanty, as shown in the preceding record of the experiments; this means slow elimination of caffein and its products of decomposition. It is conceivable that the presence of toxic amounts of caffein in the body for a considerable length of time would induce changes that ultimately lead to the death of the animal or that morbid processes are set up by the combined action of caffein and some preexisting poison. Since some guinea pigs, however, survived the doses indicated, it is more probable that such changes would be brought about by caffein in the presence of a preexisting poison. The death of these pigs, and also of No. 137 several days later, is difficult to account for on any other theory than the one suggested. Were it not for the fact that controls, that is, animals of the same lot which had not received caffein survived all of the experimental animals, changed conditions of environment or accident might be considered the cause of death in the guinea pigs of the last series.

TABLE 6.—*Subcutaneous injecticn of guinea pigs.*

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
			SERIES A.				
	Grams.	*Gram.*					
18	500	0.300	2 minutes	2 hours 40 minutes	Carrots	March	Female.
15	548	.290		30 minutes	Oats	do.	Do.
14	442	.360	15 minutes	do.	do.	do.	Do.
			SERIES B.				
20	497	0.200	2 hours 20 minutes	3 hours 30 minutes	Oats	April	Female.
38	570	.210	25 minutes	2 hours 15 minutes	Carrots	February	Male.
37	820	.200	1 hour 25 minutes	Less than 18 hours	Carrots and oats.	do.	Do.
13	618	.194		25 hours	Oats	March	Female.
36	850	.200	1 hour 30 minutes	18 hours	Carrots and oats.	February	Male.
			SERIES C.				
66	510	0.200	None	5 days	Oats	October	Male.
65	510	.200	do.	Survived	do.	do.	Do.
60	320	.219	1 hour 15 minutes	Within 18 hours	do.	do.	Female.
57	350	.200	do.	About 2½ days	do.	do.	Do.
68	785	.200	25 minutes	Less than 22 hours	do.	do.	Male.
69	585	.200	20 minutes	9 days	do.	do.	Do.
61	330	.240	1 hour 30 minutes	Less than 24 hours	do.	do.	Female.
62	335	.238	1 hour	do.	do.	do.	Do.
70	545	.238	50 minutes	About 2 days	do.	do.	Male.
71	540	.240	45 minutes	do.	do.	do.	Do.
72	560	.232	35 minutes	About 3 days	do.	do.	Do.
			SERIES D.				
49	510	0.160	1 hour 40 minutes	Less than18 hours	Oats	March	Male.
40	630	.158		Less than 2 days	Oats and carrots.	February	Do.
45	435	.160	None	Survived	Oats	March	Female.
39	820	.150		2 days	Oats and carrots.	February	Male.
41	660	.150		Survived	do.	do.	
46	470	.170	1 hour 20 minutes	do.	Oats (?)	March	Female.
			SERIES E.				
19	556	0.100		Survived	Oats	April	
42	490	.120	None	do.	do.	February	
43	430	.116	do.	do.	do.	do.	
44	535	.112	do.	do.	do.	do.	
97	330	.100	do.	do.	do.	November	
98	520	.100	do.	About 3 days	Carrots	do.	

TABLE 7.—*Injection into peritoneal cavity; guinea pigs.*

SERIES A.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Gram.*					
41	700	0.130	2 hours	22 hours	Oats	April	Male.
49	370	.135	2 hours 15 minutes.	Survived	do	do	Do.
47	550	.127	1 hour	do	do	March	Female.
50	290	.138	2 hours	do	do	April	Do.

SERIES B.

51	415	0.144	None	Survived	Oats	October	Female.
52	450	.155	do	do	do	do	Male.
53	490	.163	do	do	do	do	Do.

SERIES C.

59	375	0.200	15 minutes	Survived	Oats	October	Gray and white.
58	380	.200	10 minutes	do	do	do	
56	440	.200	15 minutes	14 days	do	do	Male.
55	690	.188	1 hour	3 hours 30 minutes	do	do	Do.

SERIES D.

67	330	0.240	5 minutes	30 minutes	Oats	October	Male.
63	340	.235		Survived	do	do	Do.
64	305	.250	25 minutes	4 hours 40 minutes	do	do	Female.

TABLE 8.—*Caffein by mouth; guinea pigs.*

SERIES J.

Number of pig.	Weight.	Caffein per kilo.	Appearance of symptoms.	Duration of life.	Diet.	Month.	Remarks.
	Grams.	*Gram.*					
129	855	0.280	40 minutes	Less than 8 hours	Hay and oats	June	Male.
130	800	.300	30 minutes	Less than 18 hours	do	do	Do.
131	860	.280	20 minutes	do	do	do	Do.
135	955	.150	1 hour 50 minutes	6 days	do	do	Do.
134	740	.160	3 hours	4 days	do	do	Do.
[1] 137	925	.150	50 minutes	6 days	do	do	Do.
[1] 136	1,000	.150	do	Survived	do	do	Do.
126	980	.200	20 minutes	27 hours	do	do	Do.
127	760	.200	1 hour	28 hours	do	do	Do.
128	1.075	.200	3 hours	7 hours	do	do	Do.

[1] Subcutaneous injection for comparison.

SUMMARY.

A survey of the results obtained in experiments on guinea pigs shows that the mode of introduction of caffein exerts but little influence on its toxicity. On careful analysis it will be observed that the rate of absorption after the administration of caffein by mouth, subcutaneously, or intraperitoneally is about the same for the time of appearance of symptoms. The persistence of the symptoms of caffein intoxication observed in these experiments for 24 hours after administration points to slow elimination, which may be expected, owing to the fact that the guinea pigs passed but little urine and caffein is not diuretic for these animals. The prolonged presence of caffein in the body probably exerts a harmful influence or after effect, which may account for the delayed death of some animals many days after a single dose of caffein was given. Among the factors which undoubtedly influence toxicity, season should be considered, while the presence of a diseased condition undoubtedly tends to decrease the resistance of the guinea pig to caffein. Diet was without any influence on the toxicity of the single dose of caffein.

EXPERIMENTS ON CATS.

These experiments were performed on well-fed animals which were kept under observation for several days before the tests with caffein were made. The diet consisted of meat exclusively. In some cases the urine was examined for albumin and sugar before caffein was given. No tests with caffein were made if large amounts of albumin were found. It may be remarked that sugar was never found in cats before the administration of caffein, but that considerable amounts of it were found in some cases after it was given. Studies by various modes of administration were made, by subcutaneous injection, intraperitoneally, or by mouth. Attention was also directed to the resistance to caffein in young cats, several experiments on kittens being made with this object in view.

SUBCUTANEOUS INJECTION.

Rost stated that caffein is eliminated in the urine unchanged after its introduction into the body and that the amounts found varied with different species of animals. In the rabbit the amount eliminated was about 21 per cent; in the dog about 8 per cent; and in the cat somewhat less than 2.5 per cent. It would appear, therefore, that the cat decomposes caffein more readily than the rabbit or dog; its resistance consequently ought to be greater than that of the other animals. Moderately large doses were accordingly employed in the preliminary experiments (series A), but the results obtained, as shown in the protocols, indicated that caffein is fully as toxic for the cat as for the rabbit or dog. The doses were then decreased and experiments were performed in order to ascertain the smallest toxic as well as the smallest fatal dose.

Series A

Three decigrams of caffein per kilo were administered in these experiments. The results are shown in the following protocols:

Cat 4. Black and white. Weight, 1,440 grams.

May 26: 10.05 a. m., 22 cc 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 11.10 a. m., copious salivation, cat irritable, muscular stiffness present, but no tetanus; 11.45 a. m., cat restless, convulsions, attacks of short duration, no paralysis observed after the convulsions, pupils dilated; 4.45 p. m., cat quiet, slight paralysis present.

May 27: Cat exhausted.

May 28: Found dead.

Cat 5. Black and white male. Weight, 1,396 grams.

June 3: 10 a. m., 21 cc of 2 per cent caffein (0.3 gram per kilo) injected subcutaneously; 12 noon, found dead.

Although there was considerable difference in the duration of life following the injection of the same dose of caffein per kilo, the final outcome was the same, as both cats died from the effects of the drug. One died within 2 hours and the other lived more than 30 hours after its administration. Three decigrams of caffein per kilo is, therefore, surely fatal to these animals. Tests made with smaller doses are shown in the following experiments:

Series B.

In these experiments the doses employed ranged between 0.20 and 0.25 gram caffein per kilo.

Cat 3. Black and white female. Weight, 2,854 grams. Well fed.

June 4: 10.30 a. m., 35 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously; 11 a. m., found dead.

Cat 6. Black and white. Weight, 1,645 grams.

June 3: 20 cc 2 per cent caffein (0.243 gram per kilo) injected subcutaneously at 3 p. m., cat grew very irritable in a few minutes; about 4. p. m. reflexes decidedly increased; 5 p. m., cat paralyzed.

June 4: Cat found dead.

Cat 8. Weight, 1,735 grams.

October 7: 4 p. m., 22 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.30 p. m., cat irritable, salivation profuse, convulsions; died at 5.30 p. m.; no urine passed after caffein was given.

Cat 9. Weight, 1,960 grams.

October 7: 3.45 p. m., 25 cc 2 per cent caffein (0.25 gram per kilo) injected subcutaneously in the back; 4.45 p. m., cat very irritable, repeated attacks of convulsions, salivation copious; died at 5.30 p. m.; cat did not urinate after injection of caffein.

Cat 12. Striped kitten. Weight, 1,185 grams.

October 9: Urine examined, no albumin, no sugar; 1.45 p. m., 12 cc 2 per cent caffein administered; 5 p. m., cat alive, no symptoms except salivation and general irritability.

October 10: 10.30 a. m., found dead. About 15 cc urine collected, but no examination made.

Cat 14. Black. Weight, 1,855 grams.

October 8: 1.40 p. m., 18.5 cc 2 per cent caffein (0.2 gram per kilo); 3 p. m., cat became restless about 10 minutes after caffein was injected; cried persistently and moved about in cage, no convulsions, cat urinated about 15 cc, cat defecated.

October 9: 9 a. m., cat found dead in cage. Urine gave very heavy reduction of Fehling's solution (much more than was obtained from urine of rabbits); 20 cc urine analyzed contained 4.65 per cent sugar. *Autopsy:* Lungs deeply congested; liver marked fatty infiltration and degeneration; spleen normal; kidneys pale and anemic; intestines normal; stomach normal.

Cat 15. Striped. Weight, 2,145 grams.

October 8: 2 p. m., 22 cc (0.2 gram per kilo) 2 per cent caffein injected subcutaneously; 2.30 p. m., cat irritable, restless, trying to get out of cage, crying persistently; 2.40, convulsions lasting about two minutes, then cat raised itself and made attempts to get out of cage, no salivation, cat urinated about 10 cc and defecated.

October 9: 9 a. m., cat found dead in cage, about 10 cc of urine contained enormous quantities of sugar. *Autopsy:* Lungs severely congested; liver showed marked fatty degeneration; spleen normal; kidneys slightly pale and anemic; intestines mildly congested; stomach normal.

Cat 19. White. Weight, 1,100 grams.

October 20: 13 cc of 2 per cent caffein (0.236 gram per kilo). About 15 minutes later cat became irritable, reflexes increased, persistent crying, stiffness of extremities, diarrhea present; 4.30 p. m., stiffness of muscles, coordination much disturbed, walked with great difficulty; 4.30 p. m., no new symptoms, persistent crying continued.

October 21: Found dead.

Cat 20. White kitten. Weight, 790 grams.

October 20: 11.35 a. m., 10 cc 2 per cent caffein (0.25 gram per kilo) given subcutaneously; 12 noon, convulsions followed by paralysis; 1.30 p. m., still breathing, apparently in comatose condition, lay on its side, dyspnoea, profuse salivation; 4 p. m., convulsions and death.

The results of the experiments of series B show that a dose of even 0.2 caffein per kilo is very toxic for the cat. Symptoms appeared in one animal 40 minutes after the injection of caffein. Some of them were found dead 18 hours after injection, which means that the duration of life was probably a great deal less since there was evidence that they had been dead for some time. Death occurred quite soon after larger doses were injected. Cat No. 3 died 30 minutes after it received caffein. The amounts employed in these experiments can not be considered therefore as the minimum fatal doses. Smaller doses were then tried, as shown in the experiments of the next series.

SERIES C.

Experiments were performed on five cats which received from 140 to 155 mg per kilo, as follows:

Cat 24. Striped. Weight, 1,300 grams.

October 25: 10 a. m., 50 cc urine, albumin moderate amount—no sugar; 10 cc caffein injected subcutaneously at 12 noon; 12.30, irritable, cried persistently, no appetite; 4 p. m., no convulsions, but persistent crying.

October 27: Cat was still alive.

Cat 17. Weight, 2,620 grams.

October 12: 9.30 a. m., 65 cc urine collected; more than a trace of albumin present, no reduction of Fehling's solution; 3 p. m., 20 cc 2 per cent caffein (150 mg per kilo) injected subcutaneously; 3.15 p. m., irritable and restless.

October 13: 9 a. m., about 15 cc urine collected, reduction of Fehling's solution marked; osazone test also positive.

Cat 23. Black and white. Weight, 1,645 grams.

October 25: 10 a. m., 140 cc urine collected (since October 23), small amount of albumin present, no sugar.

October 27: 9 a. m., no albumin; no sugar in urine; 11.50 a. m., 12.5 cc caffein injected subcutaneously (0.15 gram per kilo); 1 p. m., convulsions and death.

Cat 7. Striped kitten. Weight, 1,285 grams.

October 11: Urine collected, no albumin, no sugar; 9.50 a. m., 10 cc 2 per cent caffein injected subcutaneously in the back; 10.10, violent convulsions lasting about 30 seconds; 10.20, convulsions of shorter duration; 10.30 convulsions; 10.35, convulsions lasting a few seconds; urine passed about 10.20, contained a moderate amount of albumin, but there was no reduction of Fehling's solution; 10.45, profuse salivation and paralysis; died about 10.50.

Cat 39. Yellow. Weight, 2,285 grams.

April 13: 2.40 p. m., 16 cc 2 per cent caffein (0.14 gram per kilo) injected subcutaneously in the back; 3.45 p. m., cat died.

Of the five experiments of this series three died after doses of 140, 150, and 155 mg per kilo. The other two showed symptoms of toxicity, but survived a dose of 150 mg per kilo which indicated that the minimum fatal dose was probably reached. To test this supposition smaller doses were administered, as shown in the following experiments.

SERIES D.

Ten cats were used for this series of experiments, and the doses administered varied between 103 and 139 mg per kilo. The results shown in the appended table (p. —) indicate that about 120 to 140 mg of caffein per kilo may induce mild symptoms in some cases. The conclusion may be safely drawn therefore that 150 mg per kilo is approximately the minimum fatal dose for the cat when the drug is given subcutaneously. That smaller doses are, however, by no means to be regarded as always safe is shown in the following experiments.

SERIES E.

Cat 43. Weight, 3,225 grams.[a]

September 14: 10.20 a. m., 20 cc 2 per cent caffein (0.124 gram per kilo) injected into the back; 11 a. m., tetanus and death. *Autopsy:* Lungs congested; liver congested and showed hemorrhagic spots in capsules and fatty degeneration; kidneys slightly congested; other organs normal.

Cat 48. Black female. Weight, 3,050 grams.

September 14: 18 cc 2 per cent caffein (0.118 gram per kilo) injected subcutaneously in the back; 10.30 a. m., violent convulsions and death. *Autopsy:* Lungs congested in spots showing numerous petechia; liver congested; spleen congested; other organs normal.

The diminished resistance to caffein of cats Nos. 43 and 48 might be due to the pathologic changes found on autopsy, for evidence is not wanting that the toxicity of drugs might be greatly altered under pathological conditions. Hunt[49] has shown that resistance to acetonitril is considerably diminished in chronic alcoholism. This seems to be true also of other drugs under abnormal conditions. Smaller doses of atropin[78] are required in lead poisoning than under normal conditions to produce the same results. The following experiment is of interest in this connection, for in this case a much smaller dose than was given in experiments Nos. 43 and 48 produced the typical symptoms of caffein poisoning and proved to be fatal.

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[a] Cat probably old; had been in the laboratory for several weeks before the experiment. Gained in weight 175 grams.

Cat 47, black and white male. Weight, 4,220 grams.
September 15: Received subcutaneously 18 cc 2 per cent caffein (0.084 gram per kilo); no symptoms observed for about six hours.
September 16: No symptoms.
September 17: Weight, 4,250 grams; injected 18 cc 2 per cent caffein (0.084 gram per kilo); tetanus and death after two hours. *Autopsy:* Severe hemorrhagic pneumonia; kidneys pale, other organs normal.

Since two controls survived the same dose in proportion to the body weight of the animal without showing any symptoms, the assumption is justified that the lower resistance to caffein was due to the presence of pneumonia, thus affording additional support to the view that the toxicity of caffein may be increased in disease.

INJECTION INTO THE PERITONEAL CAVITY.

These experiments were carried out on full-grown and on young subjects. As in previous experiments, doses of different sizes were employed. A dose of 0.2 gram per kilo was tried first and then reduced gradually to 0.1 gram per kilo.

Cat 10. Female. Weight, 2,970 grams.
October 9, 1909: 1.30 p. m., 30 cc 2 per cent caffein (0.2 gram per kilo) injected into the peritoneal cavity; urine examined for albumin and sugar, negative; cat found dead at 2.30 p. m. No urine in the bladder.

Cat 16. Black female. Weight, 2,420 grams.
October 9, 1910: Urine examined for albumin and sugar, negative; 2.30 p. m., 22 cc 2 per cent caffein (0.183 gram per kilo) injected into the peritoneal cavity; found dead at 3 p. m.

Cat 99. Well-fed gray female. Weight, 3 kilos.
June 22, 1911: 3.40 p. m., 15 cc 2 per cent caffein injected into peritoneal cavity; salivation and marked irritability within one hour after injection.
June 24: Alive, appetite good.

Cat 98. Well-fed black male. Weight, 4,100 grams.
June 22: 3.45 p. m., 20.5 cc 2 per cent caffein (0.1 gram per kilo) injected into peritoneal cavity; very irritable a few minutes after injection, no other symptoms.
June 24: No symptoms, appetite good.

Cat 93. Black and white. Weight, 1,450 grams.
June 22: 3 p. m., 30 cc 2 per cent caffein (0.137 gram per kilo) injected into peritoneal cavity; salivation, no other symptoms; under observation until 6 p. m.
June 23: 9 a. m., no urine, cat showed no symptoms.
June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 87. Well-fed white female. Weight, 2,615 grams.
June 23: 2.45 p. m., 19 cc 2 per cent caffein (0.145 gram per kilo) injected into peritoneal cavity; became irritable and restless.
June 24: 9 a. m., no symptoms, took nourishment as usual.

Cat 97. Gray. Age, 3 months. Weight, 500 grams. Diet, meat.
June 24: 2.25 p. m., 5 cc 2 per cent caffein (0.2 gram per kilo) injected into peritoneal cavity; 4 p. m., no symptoms.
June 29: Died.

Cat 96. Gray and white. Age, 3 months. Weight, 575 grams. Diet, meat.
June 24: 2.20 p. m., 4 cc 2 per cent caffein (0.139 gram per kilo) injected into peritoneal cavity; 3.55 p. m., no symptoms.
June 30: Died.

Cat 95. Black. Age, about 3 months. Weight, 860 grams. Diet, meat.
June 24: 10.15 a. m., 8.6 cc 2 per cent caffein injected into peritoneal cavity, salivation immediately after injection; 10.25, convulsions and paralysis; died 10.45 a. m. *Autopsy:* Macroscopical examination of the organs, negative.

Cat 94. Black and white. Weight, 790 grams. Age, about 3 months. Diet, meat.
June 24: 10 a. m., 8 cc 2 per cent caffein injected into peritoneal cavity; 4 p. m., under continual observation since injection, cat very irritable, respiration more rapid than normal, diarrhea present.

Examination of the above protocols show that a dose of 2 decigrams per kilo was fatal within one hour to one cat and that a somewhat smaller dose killed another individual in 30 minutes. Amounts under 0.15 gram per kilo were just sufficient to induce mild symptoms, such as increased irritability and salivation, which disappeared within a few hours. In no case were the effects noticeable on the following day. The experiments on young kittens are especially interesting, as they proved, contrary to expectation, to be distinctly more resistant than full grown individuals. The death of Nos. 97 and 96 within five and six days, respectively, can not be ascribed to caffein, since some of the controls also died. Moreover, it will be remarked in this connection that no symptoms appeared in three of the four young kittens after the administration of a dose which was rapidly fatal to adult cats. The rapid death of No. 95 after the same dose forms an exception which can not be accounted for, as macroscopical examination at autopsy proved negative.

ADMINISTRATION BY MOUTH.

Two decigrams per kilo were given at first, but it was found that this amount was surely fatal. The dose was therefore reduced to 0.125 gram per kilo. In all of these experiments caffein was given by means of a soft rubber catheter slipped over the stem of a funnel which served as a stomach tube. A 2 per cent aqueous solution was used throughout these tests except in one case in which caffein was given mixed with the food.

Cat 92. Black and white female. Weight, 1,750 grams.
June 10: 12.05 p. m., 14 cc 2 per cent caffein (0.16 gram per kilo) given by mouth; cat was quiet when tied on holder, struggled only a little when tube was put into stomach; 12.30 p. m., cat vomited, no other symptoms.
June 13: Condition good, appetite good.

Cat 87. White female. Weight, 2,620 grams. Diet, meat.
June 5: 2.15 p. m., 20 cc 2 per cent caffein (0.15 gram per kilo) solution administered by mouth through stomach tube; 2.30 p. m., cat irritable, but no other symptoms; 5 p. m., condition about the same, except that it was more irritable and showed some stiffness of the extremities.
June 13: Alive and in good condition, appetite good, not irritable.

Cat 91. White female. Weight, 3,050 grams.
June 10: 12 noon, 23 cc (0.15 gram per kilo) of 2 per cent caffein administered by mouth, cat struggled violently; 1.30, salivation; 1.40 p. m., convulsions; died at 2 p. m. *Autopsy:* Congestion of lungs, liver, and spleen; heart vessels injected; other organs normal.

Cat 88. Black and white female. Weight, 3,260 grams. Diet, meat.
June 5: 2.20 p. m., 25 cc of 2 per cent caffein (0.15 gram per kilo) given by mouth; 2.45 p. m., cat irritable, no other symptoms (cat did not vomit after the administration of caffein); 4 p. m., cat found dead. *Autopsy:* Liver very much congested; heart contracted; body was still warm at the time of autopsy.

Cat 90. White and yellow female. Weight, 2,685 grams. Diet, meat.
June 5: 3.15 p. m., 27 cc of 2 per cent caffein (0.2 gram per kilo) given by mouth through stomach tube, about half an hour later cat became irritable and began to salivate; at 4.30 p. m. salivation became more marked, dyspnœa was well developed, and the cat was quite restless and had tremors; 5 p. m., short spasms of posterior extremities, but lay quietly in the cage most of the time; 5.20 p. m., convulsions of short duration and death, muscular relaxation followed immediately after convulsions, no vomiting, diarrhea observed after administration of caffein, and cat passed about 10 cc of urine.
June 6: 9 a. m., found dead.

Cat 89. White and black female. Weight, 2,860 grams. Diet, meat.
June 5: 3.15 p. m., 28.6 cc (0.2 gram per kilo) of 2 per cent caffein given by mouth through stomach tube, no vomiting observed, nor any other symptoms; 3.30, found dead. *Autopsy:* Organs normal; liver congested.

Cat 82. Gray female. March 3, weight 2,450 grams; June 6, weight 2,750 grams. Diet, 150 grams of meat daily.

June 7: Given 0.4125 gram of caffein in 150 grams of meat, did not eat.
June 8: Given 0.4125 gram of caffein in 150 grams of meat, refused to eat.
June 9: Given 150 grams of meat without caffein, ate all of it.
June 10: No food given.
June 11: No food given.
June 12: Given 0.4125 gram caffein in 150 grams of meat (150 mg per kilo), ate all of it.
June 13: Found dead. *Autopsy:* Lungs congested, liver congested; other organs apparently normal.

Cat 100. Gray female. Weight, 2,740 grams. Diet, meat.

July 17: 3 p. m., 17 cc of 2 per cent caffein (124 mg per kilo) given by mouth through stomach tube at 3.20 p. m.; 5 p. m., very irritable, but no other symptoms.
July 18: Under observation all day, no symptoms.

Cat 93. Black and white female. Weight, 1,640 grams.

July 17: 3.30 p. m., 10 cc (0.125 gram per kilo) of 2 per cent caffein given by mouth through stomach tube.
July 18: Under observation all day, no symptoms.

From the results of the experiments of this series it appears that 0.15 gram caffein per kilo may be fatal within a few hours after its administration, even if the drug is mixed with a moderate amount of meat. Experiments 87 and 92 show, however, that this amount may be borne by some individuals without any serious consequences, as the cats were under observation for some time after they received caffein, and no untoward symptoms were noticed in either of them during this time. It may be remarked that cat No. 92 vomited shortly after it received caffein. It is practically certain, therefore, that this amount of caffein in proportion to the weight of the animal will in the great majority of cases prove fatal, and perhaps in a smaller percentage of individuals it is surely toxic if it does not escape absorption. Smaller doses may cause irritability in some individuals, but symptoms referable to nervous symptoms of muscles were absent, as in experiments Nos. 93 and 100. The minimum fatal dose of caffein for the cat when given by mouth is, therefore, 0.15 gram per kilo.

TABLE 9.—*Subcutaneous injection; cats.*

SERIES A.

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
4	1,440	0.30	65 minutes	Over 29 hours.
5	1,396	.30		About 2 hours.

SERIES B.

3	2,854	0.25		30 minutes.
6	1,645	.243	Few minutes	1 hour 30 minutes.
8	1,735	.25	30 minutes	1 hour.
9	1,960	.25	1 hour	1 hour 45 minutes.
12	1,185	.20	3 hours	Less than 18 hours.
14	1,855	.20	1 hour 20 minutes	Do.
15	2,145	.20	40 minutes	Do.
19	1,100	.236	15 minutes	Do.
20	790	.25		4 hours 30 minutes.

SERIES C.

24	1,300	0.153	1 hour	Survived.
17	2,620	.15	15 minutes	Do.
23	1,645	.15		1 hour 10 minutes.
7	1,285	.155	20 minutes	1 hour.
29	2,285	.14		65 minutes.

TABLE 9.—*Subcutaneous injection; cats*—Continued.

SERIES D.

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
13	730	0.139	Restlessness	
21	1,165	.138	None	
25	965	.103	do	
26	1,605	.125	do	
27	1,625	.125	do	
28	2,335	.128	do	Received 2 doses; survived.
40	2,710	.129	do	Do.
41	1,785	.123	do	Do.
42	2,315	.112	do	Do.
38	2,325	.120	Mild	Died after second dose.

SERIES E.[1]

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
43	3,225	0.124		40 minutes.
48	3,050	.118		Died soon after.
47	4,220	.084		Survived.
[2] 47	4,250	.084		2 hours.

[1] Pathological conditions.

[2] Two days after first injection.

TABLE 10.—*Injections into peritoneal cavity; cats.*

Number.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
99	3,000	0.100	Mild	Survived.
98	4,100	.100	do.[1]	Do.
93	1,450	.137	Very mild	Do.
87	2,615	.145	do	Do.
97	505	.200	None	5 days.
96	575	.139	do	6 days.
95	860	.200	15 minutes	30 minutes.
94	790	.200	Diarrhea	Survived.
10	2,970	.200		1 hour.
16	2,420	.183		30 minutes.

[1] In few minutes.

TABLE 11.—*Administration of caffein by mouth; cats.*

No.	Weight.	Caffein per kilo.	Symptoms.	Duration of life.
	Grams.	*Gram.*		
91	3,050	0.15	1 hour 40 minutes	2 hours.
88	3,260	.15		1 hour 40 minutes.
92	1,750	.16	25 minutes	Survived.
87	2,620	.15	3 hours	Do.
90	2,685	.20	1 hour 15 minutes	Less than 18 hours.
89	2,860	.20		75 minutes.
82	2,450	.15		Less than 24 hours.
100	2,740	.124	1 hour 40 minutes	Survived.
93	1,640	.125		Do.

SUMMARY.

The toxicity of caffein in cats is shown to be the same when given by mouth as when injected subcutaneously, the minimum fatal doses in both cases being 0.15 gram per kilo. When introduced by the intraperitoneal route, caffein is, on the contrary, distinctly less toxic. After the administration of 0.137 and 0.145 gram caffein per kilo (Nos. 93 and 87) salivation in one cat (No. 93) and irritability and muscular stiffness in the other were the only effects noticed. These symptoms were no longer observed the next day and the cats appeared to be perfectly normal. Experiments with larger doses indicate that the minimum fatal dose by this method of administration is about 0.2 gram per kilo.

EXPERIMENTS ON DOGS.

The experiments were carried out on well-fed adult dogs and on puppies, kept under observation for some time before the drug was administered. Only those manifesting no signs of abnormality were used for these tests. Caffein was given by mouth mixed with 10 to 20 grams of meat, or subcutaneously in 2 per cent aqueous solution. The young animals received caffein dissolved in milk. The determination of the minimum toxic or fatal doses when the drug was fed presented considerable difficulty, as in many instances the ingestion of the drug was closely followed by vomiting.

ADMINISTRATION BY MOUTH.

SERIES A.

The effective dose in these experiments showed considerable variation. One dog (No. 38) died after a dose of 0.12 gram caffein per kilo, while some subjects survived doses of 0.2 and 0.23 gram per kilo. In the 12 experiments given in Table 12, page 62, it will be noticed that from 0.12 to 0.152 gram per kilo proved fatal to three dogs, while three others survived the same amounts in proportion to the body weight. The results were the same with larger doses. It may be observed in this connection that in the case of the five dogs in which vomiting was noticed some time during the 24 hours following the administration of caffein, four survived, No. 38 being the exception. The greater toxicity of caffein in this case is in all probability due to some morbid process, the presence of which was indicated by the high temperature of this subject.

That vomiting may avert a fatal issue after larger doses of caffein is made further probable by experiment on dog No. 48, for which, in the absence of vomiting, a dose of 0.2 gram of caffein per kilo proved fatal. On this supposition the discrepancy in the results obtained in this series may be readily explained. The smallest doses which proved fatal in these experiments were 0.145 and 0.152 gram per kilo. No. 38, which died from a dose of 0.12 gram per kilo, may be considered as an exception, as this subject was not normal. Experiments with caffein on dogs were made at various other times in this laboratory but failed to show that smaller doses of caffein, even when vomiting did not occur after its administration, were fatal, although toxic effects were observed. The conclusion is therefore justified that the minimum fatal dose of caffein for the normal dog is about 0.15 gram per kilo when given by mouth.

SUBCUTANEOUS INJECTION.

SERIES B.

To determine the toxicity of caffein more accurately, especially for comparison with animals of other species, the subcutaneous method of administration was also used. The injections were made with a syringe of 20 cc capacity, the contents of which were introduced into contiguous areas. The results of experiments on six dogs indicate that approximately 150 to 160 mg per kilo is the minimum fatal dose, since such doses proved fatal to two out of the three animals receiving this amount, while three others which received doses of from 143 to 160 mg per kilo survived.

EXPERIMENTS ON PUPPIES.

SERIES C.

In these experiments the resistance of young growing puppies to caffein was studied. Caffein was given by mouth to all the subjects except one, to which it was administered subcutaneously. The protocols, only a few of which are given, and the tabulated data of the experiments (p. 62) show that the age of the animal has a decided influence on the toxicity of caffein.

Dog 11. Weight, 1,260 grams.

August 2: At 10 a. m. given 12.5 cc of 2 per cent of caffein through stomach tube; 2 p. m., had convulsions, diarrhea, salivation, and stiffness of limbs.

August 3: Found dead 9 a. m. *Autopsy:* Thoracic viscera apparently normal; stomach immensely distended and filled with a white, cheesy mass and some fluid; round worms plentiful in stomach and small intestine; mucosa of entire intestine congested; contents of lower intestine congested; liver pale; spleen flabby; kidney congested.

Dog 10. Weight, 1,650 grams.

July 26: 9.30 a. m., 29 cc of 2 per cent caffein added to 60 cc of milk offered, but refused, and was therefore fed by mouth through stomach tube; 10.25 a. m., no symptoms; 11.30 a. m., restlessness, extremities stiff, post. extremities spread apart, dog shows well-marked symptoms of caffein poisoning; 12.10 p. m., symptoms more severe, extremities extended and spread out, is lying flat on belly so that nose touches floor of the cage; 12.40 p. m., found dead; was alive at 12.10 p. m. *Autopsy:* Lungs showed hemorrhagic foci in all lobes; heart apparently normal; liver fatty; stomach and intestines filled with round worms; spleen and kidney apparently normal.

Dog 9. Weight, 3,000 grams.

July 25: 350 mg caffein per kilo; 5 p. m., lying down most of the time, occasionally walks about in stall; restlessness present, but not marked; 5.30 p. m., vomit which looked frothy and mucilaginous noticed on the floor of the stall; no meat particles noticed in vomit, though searched for; whines occasionally.

July 26: 9 a. m., looks well; no signs of the effect of caffein given the day previous.

Dog 8. Yellow female. Weight, 3,100 grams.

July 22: 10.50 a. m., received 1.1 grams of caffein in 10 grams of meat (354 mg caffein per kilo); 3 p. m., vomited mucus; gait clumsy; refused to eat; continually drinking water; very restless; 4 p. m., convulsions set in at 3.55 p. m.; tonic rigidity of the posterior extremities; profuse salivation; convulsions were both tonic and clonic in character, and resembled those seen in rabbits in caffein intoxication; a striking feature was the duration of the spasm, which began at 3.55 p. m. and kept up for more than two hours.

July 23: Found dead at 9 a. m.

The data recorded in the table and in the protocols of the experiments of series C show that four out of the seven animals experimented upon died in less than 24 hours after caffein was fed; three of these received 300 to 354 mg caffein per kilo, and one received 200 mg caffein per kilo. No. 8 vomited four hours after caffein was given. No vomiting was observed in the other three dogs. From 0.300 to 0.350 gram of caffein per kilo may be regarded, therefore, as surely fatal to young growing puppies. That this is in all probability the minimum lethal dose appears from the following experiments: No. 9, which received 350 mg per kilo, vomited one hour after and survived, which indicates that some of it was probably not absorbed. The amount which entered the circulation was therefore less than 350 mg per kilo. Since No. 15, which received 250 mg caffein subcutaneously, likewise survived, the probabilities are that 300 to 350 mg per kilo were the minimum fatal doses for these animals. Moreover, No. 12, which received 200 mg caffein per kilo, survived, no vomiting having been observed. The case of No. 11, in which the same amount of caffein in proportion to body weight proved fatal, may be explained perhaps by the findings of the autopsy.

The results obtained in these experiments justify the conclusion that young growing dogs can stand larger doses of caffein than full-grown and older dogs.

Attention may also be called here to the difference in the symptoms produced by caffein in very young and in adult dogs. It was often noticed in these experiments that the symptoms in older subjects when given toxic doses of caffein set in rather abruptly and ended in instantaneous death. We failed to observe this phenomenon after the administration of large amounts of caffein to very young dogs, in which tonic and clonic convulsions alternating with paresis were observed. These symptoms set in rather gradually and lasted several hours (see experiment No. 8), resembling the rabbit in this regard.

SUMMARY.

The toxicity of caffein for adult dogs is about the same, whether given by mouth or injected subcutaneously. The resistance of puppies to caffein is much greater than that of adults.

TABLE 12.—*Administration by mouth; dogs.* (*Series A.*)

No.	Weight.	Caffein per kilo.	Results.	Remarks.
	Kilos.	*Gram.*		
47	13.60	0.144	Survived	Vomiting observed.
55	12.75	.200	.....do	Stiffness of muscles; no other symptoms.
56	7.95	.200	Found dead next day	
52	13.60	.147	Survived	
57	6.50	.230	.....do	Vomited after 1 hour; convulsions after 1 hour and 45 minutes.
39	23.10	.120	.....do	Increased frequency of respiration, thirst, loss of appetite, vomited rest of day when he drank water, salivation, restlessness, passed feces frequently.
48	11.50	.174	.....do	Vomiting observed.
48	12.00	.200	Found dead next day	No vomiting observed. Second dose was given 8 days after first.
54	13.40	.200	Survived	Vomiting observed.
49	13.15	.152	Found dead next day	
38	14.50	.120	.....do	Symptoms after 1½ hours: Dog had a temperature of 104° F. before caffein was given; vomited 3 hours after caffein was fed.
18	10.30	.145	.....do	

TABLE 13.—*Subcutaneous injection; dogs.* (*Series B.*)

No.	Weight.	Caffein per kilo.	Results.	Remarks.
	Kilos.	*Gram.*		
62	9.30	0.161	Survived	Restlessness and vomiting one-half hour after injection.
61 A	14.00	.160	Found dead next day	
63	12.00	.150	Survived	Restlessness 1 hour after injection.
64	14.00	.150	.....do	Restlessness and thirst 45 minutes after injection.
59	7.20	.160	Died 1 hour and 20 minutes after injection.	Marked restlessness, but no convulsion.
61	14.60	.143	Survived	Symptoms observed in 1½ hours.

TABLE 14.—*Administration by mouth to puppies.* (*Series C.*)

No.	Weight.	Caffein per kilo.	Result.	Remarks.
	Kilos.	*Gram.*		
8	3.10	0.354	Found dead next day.	Vomited in 4 hours after feeding; restlessness, loss of appetite, thirst, incoordination of muscles, convulsions.
9	3.15	.350	Survived	Muscular incoordination and stiffness, restlessness, vomited 1 hour after caffein was given.
10	1.60	.350	Died in 3 hours	Convulsion; no vomiting.
11	1.26	.200	Found dead next day.	Salivation; convulsions.
12	1.28	.200	Survived	No symptoms.
15	1.20	.250	.....do	Subcutaneous injection.
16	3.50	.300	Died in 1 hour	Convulsions 45 minutes after caffein was fed.

CHRONIC CAFFEIN INTOXICATION.

The object of this study was to ascertain the effect of repeated dosage when caffein was given daily or at longer intervals. The experiments were tried on rabbits and on dogs. As in the experiments on acute intoxication, the animals were under observation for some time in the laboratory before the administration of caffein was begun in order to ascertain the presence or absence of abnormality. The relation of diet to toxicity received some attention, but the question was not studied exhaustively in the present investigation.

EXPERIMENTS ON RABBITS.

Full-grown adult as well as young rabbits were employed. The diet consisted either of carrots or of oats; water was given ad libitum. The rabbits were kept in metal cages in a well-lighted and well-ventilated room. Unnecessary handling or any other procedure tending to fatigue or to cause discomfort to the animals was very carefully avoided, since we had found that such treatment was likely to decrease the resistance of the rabbit to caffein. The caffein was administered by feeding by mouth and through a stomach tube, or by the subcutaneous method. In a good many cases it was given daily, in some at longer intervals.

SERIES A.

The experiments of this series formed a preliminary study for the purpose of testing the effect of moderate doses. One decigram of caffein per kilo was given daily for several days; when administered at longer intervals the dose was increased to 150 to 200 mg per kilo. It was found that the smaller doses did not produce any symptoms; even the weights of the animals were not influenced. Doses of medium size given on two successive days were likewise without any noticeable effect (Nos. 182, 183, 123, 101). When a third dose of this size was given within 48 or 24 hours it proved fatal (Nos. 123, 182, and 183). Exceptionally, however, moderately large doses (for rabbits) may be given for three consecutive days without fatal issue, as in rabbit No. 101. When given at intervals of two to three days, larger doses, as may be seen from the protocols, can be administered without causing acute death (Nos. 173, 181, 201).

The results of the tests of this series point to the absence of any accumulation and to the possible elimination of moderate doses of caffein and its products of decomposition within 24 hours or thereabouts. When the doses are larger the time of its elimination is apparently longer, as shown by the fact that repetitions of the dose the next day may be fatal, but when a longer interval is allowed it may be given without causing death. It will be observed that only one rabbit of this series survived, but it was extremely emaciated. This condition has been observed in a number of cases after caffein had been given for several days. Even when the drug was withdrawn the animals continued to lose weight. This may be explained by the condition of the gastro-intestinal canal as found at autopsy. The presence of inflammation of the mucous membrane of the stomach and intestines, with ulceration of the mucous membrane of the pylorus in one of the rabbits (No. 173) of the series, in all probability caused diminution or loss of appetite, which of itself would tend to cause loss of flesh and strength and finally death. Protocols of the experiments follow.

Rabbit 173. Carrots were fed from October 1 to 18 and oats for the remainder of the experiment.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,980	0.141	Oct. 16	2,005	0.220
Oct. 11	1,905	.190	Oct. 18	1,845	
Oct. 13	1,930	.207	Oct. 20	1,740	.239

October 21: Paralysis of posterior extremities.

October 22: 9 a. m., found dead.

The urine was examined before and after the administration of caffein. No symptoms were observed after the administration of caffein (5 doses in 11 days), nor was albumen or sugar found in the urine after any of the experiments on this rabbit. *Autopsy:* Pyloric mucosa exhibited several ulcers; small intestines showed slight inflammation; liver deeply congested; kidneys showed marked inflammation of cortex; other organs practically normal.

Rabbit 181. Diet, carrots September 29–October 17, then oats.

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,425	0.105	Oct. 11	1,370	0.175
Oct. 5	1,450	.100	Oct. 13	1,385	.180
Oct. 6	1,270	.100	Oct. 16	1,345	.200
Oct. 7	1,210	.100	Oct. 17	1,030	
Oct. 8	1,375	.130	Oct. 18	1,230	
Oct. 9	1,305	.153	Oct. 20	1,215	

Rabbit was markedly emaciated and weak. No albumen or sugar found in the urine as a result of caffein feeding.

Rabbit 182. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,765	0.100	Oct. 8	1,685	.135
Oct. 5	1,880	.100	Oct. 9		.150
Oct. 6	1,750	.100	Oct. 11	1,605	.174
Oct. 7	1,710	.100			

October 12: 11 a. m., 23 hours after caffein was given, convulsions with recovery; rabbit died at 1.30 p. m. No sugar was found in the urine at any time after the administration of caffein. Albumen was present only in one specimen.

Rabbit 183. Diet of carrots from September 29.

Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 4	1,385	0.100	Oct. 8	1,310	0.153
Oct. 5	1,460	.100	Oct. 9	1,390	.142
Oct. 6	1,385	.100	Oct. 11	1,390	.157
Oct. 7	1,240	.122			

October 12: 9 a. m., found dead. No albumen was found in the urine. Only one sample contained sugar.

Rabbit 123. White, female. Diet, oats.
Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Grams.*		*Grams.*	*Grams.*
Apr. 14	2,350	42	Apr. 20	2,126	141
Apr. 16	2,250	90	Apr. 21	1,965	152
Apr. 17	2,325	86	Apr. 22	1,876	160

Rabbit died 30 minutes after last injection of caffein. *Autopsy:* Stomach exhibited marked inflammation of mucosa. Slight enteritis. Liver and kidneys were deeply congested and dark colored.

Rabbit No. 101, white male. Diet, oats.
Received caffein subcutaneously as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Mar. 18	2,025	0.100	Mar. 24	1,815	0.166
Mar. 19	1,970	.100	Mar. 25	1,830	.185
Mar. 20	2,009	.100	Mar. 26	1,710	.176
Mar. 22	1,855	.100	Mar. 29	1,734	.219
Mar. 23	1,738	.114	Apr. 1	1,606	.224

April 5: Found dead. *Autopsy*: Marked inflammation of gastric mucosa. Considerable enteritis affecting the whole extent of the intestines; liver congested and friable; kidneys deeply congested in cortical and medullary portions; spleen congested, but of normal size; lungs and heart normal.

Four days, 0.1 per kilo; 10 doses in 14 days.

Rabbit 201. Diet of carrots begun October 1; October 19, oats.
Subcutaneous injections as follows:

Date.	Weight.	Caffein per kilo.	Date.	Weight.	Caffein per kilo.
	Grams.	*Gram.*		*Grams.*	*Gram.*
Oct. 9	1,000	0.150	Oct. 16	1,065	0.225
Oct. 11	1,015	.180	Oct. 18	850	....
Oct. 13	1,065	.187	Oct. 20	890	.111

Under observation six hours October 20; no symptoms.

October 23: Died; was much emaciated but did not show any symptoms; emaciation set in when caffein was withdrawn; urine never contained sugar or albumen; symptoms observed after second dose only.

SERIES B.

The question whether caffein is cumulative in the rabbit, suggested in the preceding experiments, was the subject of further investigation in Series B. Caffein was given by mouth or subcutaneously. Carrots formed the exclusive diet, a measured amount being given. The rabbits were kept under observation for two weeks, except Nos. 370 and 373, records of which were made only for four days before the administration of caffein was begun. Caffein was given by mouth in experiments of Groups I and III. Rabbits 292, 293, and 295 received daily 20 cc water by mouth for four days previous to the administration of caffein, while in the rabbits of Group II the caffein treatment was preceded by the injection of 0.8 per cent salt solution subcutaneously. The object in both cases was to ascertain whether or not the method of the administration of caffein has any influence on the animal, but observation made from day to day failed to show any effect of such treatment. About 1 decigram of caffein per kilo was administered daily, with occasional intermissions. Later in the course of the experiment the doses were increased, 0.15 gram per kilo being the maximum dose given. Rabbit 293 died after the third dose with symptoms of typical caffein poisoning. The administration of the same dose of caffein was continued 10 days longer in Nos. 292 and 295. It was omitted on the seventh, fourteenth, and fifteenth days of the experiment. On the eighteenth day of the experiment the dose was increased to 150 mg per kilo and was repeated 2 days later. No. 295 was found dead the next day. No. 292 survived. Rabbits 313 and 315 may be considered together, as they were treated alike in every respect. The initial dose of 100 mg caffein per kilo was finally increased to 122 mg. After the twelfth dose the emaciation was well marked and the rabbits were very weak. No. 313 was found dead 2 days, and No. 315 3 days, after the last dose of caffein was given. It should be remarked in this connection that symptoms of caffein poisoning were never observed in these rabbits. Death was not due, therefore, primarily to caffein, but the rapid loss of flesh and strength observed during the last few days suggests that it was due to malnutrition apparently brought about by caffein.

The results obtained by subcutaneous injection of caffein are given in the table as Group II. The initial dose of 100 mg per kilo was injected daily. No. 298 died after the second dose. Nos. 223 and 296 received this amount daily for 6 days. An intermission of 2 days followed, at the end of which the same dose was given again. The next day it was increased to 150 mg per kilo, but no effect was observed; 48 hours later this dose was repeated. No. 223 was found dead, but its mate survived. Symptoms of acute caffein intoxication were not observed in any of these rabbits. It would seem, therefore, that caffein is not cumulative. This supposition, however, appears somewhat contradictory in view of the fact that out of the eight rabbits of this series six died, nor could any cause of death be ascribed other than caffein. Also the first results of Experiments 293, 370, and 373 might be considered as indicating that cumulation, though to a moderate extent, does take place, since in these cases reflexes developed after the drug was given for some time. But this view is contradicted by the results of Experiment 371, in which 150 mg per kilo given 5 days after the daily dosage of caffein was suspended likewise caused increased reflexes. Cumulation, therefore, does not account for the effects noted in the other rabbit. It will be observed that rabbit No. 370, as well as Nos. 371 and 373, had diarrhea for several days. It is quite possible that the weakened condition rendered the rabbits more sensitive to the action of the drug. This is made highly probable by the observations recorded in the experiments on acute intoxication with caffein in which death occurred after small doses. In such cases some pathological condition was often disclosed by the autopsy. The results of this series corroborate, therefore, those of Series A, and indicate again the absence of cumulative action. The results obtained are in all probability due to malnutrition and other conditions brought about by congestion of the viscera and consequent injury to the gastro-intestinal canal.

TABLE 16.—*Chronic caffein intoxication of rabbits; Series B on cumulation.*

Data.	Group I.			Group II.			Group III.	
	No. 292.	No. 293.	No. 295.	No. 296.	No. 223.	No. 298.	No. 315.	No. 313.
iet. (grams carrots in 2 days)	1,000	1,000	975	930	905	880	355	300
ffein administered (cc) and weight (grams):								
Mar. 5	 1,410	 1,470	 1,045	 1,040	 1,070	 955	 770	 770
Mar. 7	 1,415	 1,360	 1,140	 1,090	 1,095	 1,000	 715	 690
Mar. 9	 1,350	 1,270	 1,070	 1,000	 1,055	 1,005	 655	 665
Mar. 11	 1,505	 1,465	 1,190	 1,230	 1,285	 1,250	 755	 760
Mar. 16	 1,580	 1,460	 1,230	 1,165	 1,170	 1,145	 730	 745
Mar. 17	 1,515	 1,415	 1,080	 1,040	 1,115	 1,105	 720	 685
Mar. 19	 1,565	 1,570	 1,280	 1,195	 1,235	 1,220	 710	 735
Mar. 21	7 1,585	7 1,530	6 1,265	 1,150	 1,215	 1,260	4 755	4 700
Mar. 22	7 1,440	7 1,315	6 1,175	(1) 1,100	(1) 1,045	(1) 1,150	4 675	4 635
Mar. 23	7 1,335	7 1,140	6 1,110	(1) 1,145	(1) 1,190	(1) 1,230	4 715	4 700
Mar. 24	7 1,310	...... (2)	6 1,090	(1) 1,115	(1) 1,170	(1) 1,250	4 680	4 650
Mar. 25	7 1,375	....	6 1,035	(1) 1,125	(1) 1,215	(1) 1,215	4 695	4 685
Mar. 26	7 1,255	....	6 1,095	 1,105	 1,155	 1,150	4 675	 695
Mar. 27	....	....	....	....	....	....	....	....
Mar. 28	7 1,355	....	6 1,115	5.5 1,120	6 1,160	6 1,155	4 595	4 685
Mar. 29	7 1,385	....	6 1,150	6 1,155	6 1,165	5 955	4 695	4 675
Mar. 30	7 1,330	....	6 1,075	6 1,035	6 1,095	...... Dead.	4 630	4 610
Mar. 31	7 1,325	....	6 1,170	6 1,110	6 1,140	....	4 690	4 605
Apr. 1	7 1,335	....	6 1,050	6 1,050	6 1,120	....	4 625	4 620
Apr. 2	7 1,390	....	6 1,125	6 1,090	6 1,155	....	4 695	4 625
Apr. 3	....	....	....	....	....	....	 200	....
Apr. 4	 1,300	....	 1,005	 1,105	 1,080	....	 585	 580
Apr. 5	7 1,385	....	6 1,090	6 1,130	6 1,090	....	4 655	4 630
Apr. 6	9 1,260	....	7.5 1,010	7.5 1,050	8 1,110	....	 560	 530
Apr. 7	....	....	....	....	....	....	....	...... (3)
Apr. 8	9 1,260	....	7.5 1,000	7.5 1,090	8 1,065	....	...... Dead.	....
Apr. 9	Survived.	......	Dead.	Survived.	Dead.	......	......	Dead.

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[1] On these days 5 cc of salt solution was administered subcutaneously.

[2] Dead Mar. 23.

[3] Found dead 9 a. m.

TABLE 17.—*Chronic intoxication of rabbits, series B, Group IV, on cumulation.*

RABBIT, 370.

Date.	Weight.	Carrots.	Water.	Urine.	Caffein by stomach.	Symptoms.
	Grams.	*Grams.*	*cc.*		*Mg per kilo.*	
Aug. 7	2,155	450	50	280	....	
Aug. 8	2,030	450	25	185	....	
Aug. 9	2,105	290	0	275	....	
Aug. 10	2,095	450	30	335	....	
Aug. 11	2,105	450	65	360	50	
Aug. 12	2,125	450	65	220	50	
Aug. 13	2,120	350	25	265	50	
Aug. 14	2,170	450	35	275	75	
Aug. 15	2,175	350	(?)	200	75	
Aug. 16	2,170	360	65	250	75	
Aug. 17	2,175	310	35	170	100	
Aug. 18	2,095	180	40	285	100	Severe diarrhea.
Aug. 19	2,120	400	(?)	285	125	Do.
Aug. 20	2,120	400	(?)	310	125	Better.
Aug. 21	2,120	400	70	250	125	Do.
Aug. 22	2,040	400	45	265	150	Diarrhea bad.
Aug. 23	2,030	370	35	220	150	Diarrhea better.
Aug. 24	1,950	215	40	120	150	Do.
Aug. 25	1,885	195	35	60	200	Reflexes.
Aug. 26	....	....	....	....	....	Found dead at 9.

RABBIT, 373.

Aug. 7	2,240	450	50	230	....	
Aug. 8	2,150	150	30	300	....	
Aug. 9	2,120	205	0	150	....	
Aug. 10	2,150	450	15	245	....	
Aug. 11	2,195	450	5	285	50	
Aug. 12	2,160	450	65	325	50	
Aug. 13	2,120	300	45	190	50	
Aug. 14	2,195	450	40	265	75	
Aug. 15	2,215	350	35	200	75	
Aug. 16	2,205	310	45	225	75	
Aug. 17	2,240	400	40	265	100	
Aug. 18	2,255	350	30	320	100	
Aug. 19	2,115	185	(?)	170	125	Severe diarrhea.
Aug. 20	2,115	280	35	195	125	Diarrhea better.
Aug. 21	2,050	175	75	115	125	Slight diarrhea.
Aug. 22	2,060	180	75	130	150	
Aug. 23	2,005	200	75	125	150	Reflexes.
Aug. 24	1,990	200	75	150	150	Slight diarrhea.
Aug. 25	1,950	255	55	132	175	Severe diarrhea.
Aug. 26	1,870	205	80	140	None	Do.
Aug. 27	1,830	200	50	140	do	Do.
Aug. 28	1,950	400	25	265	do	Slight diarrhea.
Aug. 29	1,825	400	0	315	do	Very weak and in poor condition.
Aug. 30	1,850	....	10	140	do	
Aug. 31	1,835	....	....	....	....	

TABLE 17.—*Chronic intoxication of rabbits, series B, Group IV, on cumulation*—Contd.

RABBIT, 371.

Date.	Weight.	Carrots.	Water.	Urine.	Caffein.	Symptoms.
	Grams.	*Grams.*	*cc.*		*Mg per kilo.*	
Aug. 7	2,240	450	50	300	....	
Aug. 8	2,260	450	50	225	....	
Aug. 9	2,310	430	(?)	300	....	
Aug. 10	2,295	450	50	305	....	
Aug. 11	2,320	450	50	335	50	
Aug. 12	2,280	450	70	400	50	
Aug. 13	2,300	350	70	255	50	
Aug. 14	2,265	425	55	154	75	
Aug. 15	2,260	250	40	125	75	
Aug. 16	2,295	155	70	Lost....	75	
Aug. 17	2,180	105	70	120	100	Severe diarrhea.
Aug. 18	2,150	125	70	100	100	Diarrhea better.
Aug. 19	2,075	210	(?)	192	100	Diarrhea severe.
Aug. 20	2,075	280	70	180	100	Do.
Aug. 21	2,165	260	50	225	None....	Diarrhea better.
Aug. 22	2,105	400	50	275	...do.....	
Aug. 23	2,080	300	0	145	...do.....	Diarrhea severe.
Aug. 24	2,105	250	15	245	...do.....	Do..
Aug. 25	2,055	320	10	176	150	Reflexes.
Aug. 26	2,040	190	75	250	150	Died at 1 p. m., without having showed any symptoms other than reflexes.

SERIES C.

The subjects used in these experiments were rabbits of medium size and were apparently young or at any rate were not very old. The series was planned for the study of the possible effect of diet on the toxicity of caffein when given for some time, and therefore oats were substituted for carrots, which had been fed in the previous work, as already stated. Caffein was given by mouth in the usual way, in 1 per cent solution, 100 mg. per kilo daily. Fourteen rabbits were used for these tests. Their weights were recorded daily and observations made at frequent intervals during the day.

The only change noticed in all of the experiments of this series was progressive loss of weight which set in from 3 to 8 days after the administration of the drug was begun. The duration of life varied considerably. No. 382 died after the first dose. No. 389 lived 2 days, No. 386, 3 days, and No. 385, 5 days, No. 390 lived 7 days and No. 404 lived 20 days after the administration of caffein was begun. The duration of life in all the others was from 11 to 16 days. The findings at autopsy are interesting and suggestive as regards the possible explanation of the effects of repeated dosage of caffein. In eight of the rabbits there was involvement of the mucous membrane of the stomach or intestines or of both. Since the same condition of the gastro-intestinal canal was observed in previous experiments with caffein when injected subcutaneously, the mere passing of the tube into the stomach is obviously not the cause of this condition. The fatal outcome due is therefore, as was suggested above, to inanition brought about by the condition of the gastro-intestinal canal. Moreover parallel experiments carried out on rabbits in the same way with alcohol survived this treatment much longer. Obviously then the passing of the soft rubber catheter is not the cause of this condition of the gastro-intestinal canal nor the diet. Rabbits were fed oats exclusively for several months in this laboratory and thrived. The presence of pneumonia in the other rabbits of this series may be regarded as accidental, as it is inconceivable that one or two doses of caffein, as was the case in Nos. 382 and 389, could predispose the lungs to infection. The results of these experiments therefore are in harmony with those of the preceding two series, indicating that caffein does not accumulate in the body, and that the toxicity of caffein, whether of the single dose or of repeated doses is the same, on a diet of carrots or of oats. These results also show that caffein is much more toxic with repeated dosage. As stated in the historical part of this bulletin the same view was held by Gourewitch.[28]

Rabbit 386. Belgian female.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		*Grams.*	cc.
Aug. 17	1,300	13.0	Aug. 19	(?)	(?)
Aug. 18	1,215	12.0			

August 20: Found dead 9 a. m. *Autopsy*: Lungs slightly congested; liver engorged and friable; gall cyst well filled.

Rabbit 389. Black male.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		*Grams.*	cc.
Aug. 17	1,070	10.0	Aug. 18	1,025	10.0

August 19: Found dead 9 a. m. *Autopsy*: Lungs severely congested and partially hepatized; liver was engorged; other organs appeared normal.

Rabbit 382. Belgian female.

On August 17 weighed 1,035 grams; received 1 cc of 1 per cent caffein for each 100 grams; 10 cc of 1 per cent caffein given in all.

August 18: Found dead 9 a. m. *Autopsy*: Lungs congested and hepatized; liver engorged; stomach showed numerous petechial hemorrhages on mucosa; kidneys slightly congested; intestines appeared normal.

Rabbit 385. Belgian female.

Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		*Grams.*	cc.
Aug. 17	780	8.0	Aug. 20	715	7.0
Aug. 18	760	7.5	Aug. 21	700	7.0
Aug. 19	755	7.5			

August 22: Found dead 9 a. m. *Autopsy*: Lungs exhibited pneumonic lesions, with inflammation of adjacent pleura; a fibro-plastic exudate present around lung; liver showed a coccidial infestation; stomach distended with ingesta; mucous membrane characterized by a catarrhal inflammation; contents of small intestine liquid in nature and bile stained; large intestine somewhat impacted; liver and kidneys seemingly normal.

Rabbit 404. White male.
Given 1 cc 1 per cent caffein for each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	1,465	14.5	Aug. 30	1,320	13.0
Aug. 21	1,475	14.5	Aug. 31	1,330	13.5
Aug. 22 [1]	....	....	Sept. 1	1,335	13.5
Aug. 23	1,475	14.5	Sept. 2	1,315	13.0
Aug. 24	1,400	14.0	Sept. 3	1,350	13.5
Aug. 25 [2]	1,405	14.0	Sept. 4	1,335	13.5
Aug. 26	1,415	14.0	Sept. 5	1,350	13.5
Aug. 27	1,400	14.0	Sept. 6	1,380	14.0
Aug. 28 [1]	....	....	Sept. 7	1,375	14.0
Aug. 29	1,310	13.0	Sept. 8	1,325	13.0

[1] Not fed.

[2] Reflexes.

September 9: Found dead 9 a. m. *Autopsy*: Both lungs showed extensive pneumonia, with adhesions to pleura; pleuritis and pericarditis very marked; large amount of fibrous exudate in pleural cavity; pyloric end of stomach slightly congested; liver congested; other organs normal.

Rabbit 393. Belgian.
Given 1 cc of 1 per cent caffein to each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	950	9.5	Aug. 25	835	8.5
Aug. 18	910	9.0	Aug. 26	780	8.0
Aug. 19	895	9.0	Aug. 27	765	7.5
Aug. 20	910	9.0	Aug. 28 [1]	....	....
Aug. 21	905	9.0	Aug. 29	710	....
Aug. 22 [1]	....	....	Aug. 30 [2]	....	....
Aug. 23	825	8.0	Aug. 31 [1]	....	....
Aug. 24	870	8.5			

[1] Not fed.

[2] Condition very poor; not fed.

September 1: Found dead. *Autopsy*: Lungs congested and adhering to the pleura; extensive inflammation of pleura; liver slightly enlarged and congested; mucosa of stomach and small intestines slightly congested; other organs normal.

Rabbit 390. Belgian, male.
Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,490	15.0	Aug. 21	1,265	12.5
Aug. 18	1,370	14.0	Aug. 22 [1]	....	....
Aug. 19	1,365	13.5	Aug. 23	1,120	11.0
Aug. 20	1,340	13.5			

[1] Not fed.

August 24: Found dead 9 a. m. *Autopsy:* Heart and lungs appeared normal; abdominal viscera showed no apparent pathologic change other than coccidial infection of the liver and fullness of the blood vessels.

Rabbit 392. Maltese, female.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,265	12.5	Aug. 26	1,140	11.5
Aug. 18	1,275	12.5	Aug. 27	1,140	11.5
Aug. 19	1,240	12.5	Aug. 28[1]		
Aug. 20	1,220	12.0	Aug. 29	1,115	11.0
Aug. 21	1,245	12.5	Aug. 30	1,080	11.0
Aug. 22[1]			Aug. 31	1,020	10.0
Aug. 23	1,180	12.0	Sept. 1	995	10.0
Aug. 24	1,190	12.0	Sept. 2	930	9.0
Aug. 25	1,155	11.5			

[1] Not fed.

Died at 3 p. m. September 2. *Autopsy:* The stomach and small intestines showed numerous small hemorrhagic spots; a thick coating of mucus surrounded the contents of the stomach; the other organs were apparently normal.

Rabbit 403. Black.

Given 1 cc of 1 per cent caffein for each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 20	1,640	16.5	Aug, 26	1,390	14.0
Aug. 21	1,640	16.5	Aug. 27	1,330	13.5
Aug. 22[1]			Aug. 28[1]		
Aug. 23	1,490	15.0	Aug. 29	1,130	11.5
Aug. 24	1,515	15.0	Aug. 30	1,055	10.5
Aug. 25	1,475	15.0			

[1] Not fed.

August 31: Found dead at 3 p. m. *Autopsy:* Extensive gastroenteritis; liver enlarged and congested; spleen slightly congested; peritoneum thickened and congested; other organs normal.

Rabbit 384. Black, female.

Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 16	1,195	12.0	Aug. 25	990	10.0
Aug. 17	1,205	12.0	Aug. 26	960	9.5
Aug. 18	1,140	11.5	Aug. 27	955	9.5
Aug. 19	1,180	12.0	Aug. 28[1]		
Aug. 20	1,145	11.5	Aug. 29	870	9.0
Aug. 21	1,145	11.5	Aug. 30[2]	850	8.5
Aug. 22[1]			Aug. 31	810	8.0
Aug. 23	1,005	10.0	Sept. 1	740	7.5
Aug. 24	1,035	10.5			

[1] Not fed. [2] Poor condition, mucus from rectum.

September 2: Found dead at 9 a. m. *Autopsy:* The mucosa of stomach showed numerous hemorrhagic spots; the first portion of the small intestines was slightly congested; the other organs were apparently normal in appearance.

Rabbit 383. Belgian, female.
Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 16	995	10.0	Aug. 22[1]	....	....
Aug. 17	1,005	10.0	Aug. 23	875	9.0
Aug. 18	990	10.0	Aug. 24	855	8.5
Aug. 19	895	9.0	Aug. 25	850	8.5
Aug. 20	945	9.5	Aug. 26	785	8.0
Aug. 21	965	9.5	Aug. 27	710	7.0

[1] Not fed.

August 28: Found dead at 9 a. m. *Autopsy:* Lungs, heart, and spleen apparently normal; liver infected with coccidia; stomach apparently normal; walls of small intestines injected; colon marked congestion and hemorrhagic; kidneys hemorrhagic.

Rabbit 387. Belgian male.
Given 1 cc of 1 per cent caffein for each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,260	12.5	Aug. 26	1,185	12.0
Aug. 18	1,340	13.0	Aug. 27	1,255	12.5
Aug. 19	1,335	13.0	Aug. 28[1]	....	....
Aug. 20	1,300	13.0	Aug. 29	1,115	11.0
Aug. 21	1,325	13.0	Aug. 30	1,135	11.5
Aug. 22[1]	....	....	Aug. 31	1,175	12.0
Aug. 23	1,205	12.0	Sept. 1	1,050	10.5
Aug. 24	1,200	12.0	Sept. 2	900	9.0
Aug. 25	1,285	12.5			

[1] Not fed.

September 3, found dead. *Autopsy:* Stomach and small intestines showed numerous hemorrhagic spots; thick coating of mucus surrounded the contents of the stomach; bladder was greatly distended with urine; the other organs were apparently normal.

Rabbit 388. Belgian male.
Given 1 cc of 1 per cent caffein for each 100 grams, through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	*cc.*		*Grams.*	*cc.*
Aug. 17	1,080	10.0	Aug. 23	1,020	10.0
Aug. 18	1,115	11.0	Aug. 24	985	10.0
Aug. 19	1,150	11.5	Aug. 25	960	9.5
Aug. 20	1,130	11.5	Aug. 26	900	9.0
Aug. 21	1,120	11.0	Aug. 27	875	9.0
Aug. 22[1]	....	....	Aug. 28[1]	....	....

[1] Not fed.

August 29, found dead 9 a. m. *Autopsy:* Heart and lungs normal; liver and kidneys engorged; stomach normal; intestines showed a catarrhal inflammation, though not severe; spleen normal; walls of colon somewhat injected.

Rabbit 391. Belgian.

Given 1 cc of 1 per cent caffein to each 100 grams through stomach tube.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		*Grams.*	cc.
Aug. 17	940	9.5	Aug. 24	805	8.0
Aug. 18	950	9.5	Aug. 25	800	8.0
Aug. 19	955	9.5	Aug. 26	765	7.5
Aug. 20	935	9.5	Aug. 27 [2]	690	7.0
Aug. 21	945	9.5	Aug. 28 [1]	....	....
Aug. 22 [1]	....	....	Aug. 29	565	5.5
Aug. 23	835	8.5			

[1] Not fed.

[2] Poor condition.

August 30, found dead 9 a. m. *Autopsy:* Heart injected; lungs normal; liver affected slightly with coccidiidea; stomach normal in appearance; small intestines normal, but colon considerably inflamed; kidneys slightly engorged; other organs normal.

Rabbit 402. Black female.

Given 1 cc of 1 per cent caffein to each 100 grams.

Date.	Weight.	Treatment.	Date.	Weight.	Treatment.
	Grams.	cc.		*Grams.*	cc.
Aug. 20	2,030	20.0	Aug. 27	1,765	17.5
Aug. 21	1,950	19.5	Aug. 28 [1]	....	....
Aug. 22 [1]	....	....	Aug. 29	1,630	16.5
Aug. 23	1,955	19.5	Aug. 30	1,540	15.5
Aug. 24	1,905	19.0	Aug. 31	1,510	15.0
Aug. 25	1,890	19.0	Sept. 1	1,425	14.0
Aug. 26	1,780	18.0			

[1] Not fed.

September 2, found dead 9 a. m. *Autopsy:* The lungs were badly congested, the posterior lobe of the right lung showing hepatization; the liver was considerably enlarged and congested; the mucous membrane of the stomach and small intestines was congested and showed numerous hemorrhagic spots; the kidneys showed slight congestion; all other organs normal.

SERIES D.

The evidence brought forth in the preceding pages regarding cumulation of caffein naturally suggests the question whether or not the body acquires a tolerance for it. This question has already been answered in the affirmative by Gourewitch,[28] but owing to the method he used for the identification of caffein and the few experiments made his results are not conclusive. The experiments of series A, B, and C might be regarded as indicating that tolerance for caffein is not acquired by the rabbit. It was noticed, however, that the rabbit apparently does tolerate increasingly larger doses under certain conditions, as the following experiments show:

Rabbit 223. Belgian hare, male.

October 22: Weight, 1,520 grams; 15 cc 2 per cent caffein injected subcutaneously at 2 p. m.

November 1: 10.30 a. m., weight, 1,510 grams; 17 cc 2 per cent caffein injected subcutaneously (225 mg per kilo), reflexes observed, but no tetanus.

November 4: 10.30 a. m., weight 1,535 grams; 19 cc 2 per cent caffein injected subcutaneously at 2.40 p. m.; 4.40 p. m., no symptoms.

November 8: Weight, 1,425 grams; 20 cc 2 per cent caffein (285 mg per kilo) injected at 11.45 p. m.; 5 p. m., no symptoms.

November 17: Weight, 1,325 grams; 22 cc 2 per cent caffein injected at 2.55 p. m. (329 mg per kilo), no symptoms.

November 18: Rabbit in good condition.

Rabbit 224. Belgian hare, female: Diet, carrots.

October 18: Weight, 1,935 grams; 11.20 a. m., 15 cc 2 per cent caffein (155 mg per kilo) injected.

November 1: Weight, 1,780 grams; 20 cc 2 per cent caffein (224 mg per kilo) injected subcutaneously, reflexes increased, muscle tremors present, but no other symptoms.

November 4: Weight, 1,710 grams; 21.5 cc 2 per cent caffein (252 mg per kilo) injected.

November 8: Weight, 1,435 grams; 22.5 cc 2 per cent caffein or 314 mg per kilo injected at 11.40 p. m.; 5 a. m., no symptoms.

November 17: Weight, 1,340 grams; 24 cc 2 per cent caffein (358 mg per kilo) injected subcutaneously.

November 18: 9 a. m., rabbit died.

Rabbit 226. Gray male. Diet, carrots.

October 28: Weight, 1,045 grams; 10 cc 2 per cent caffein injected subcutaneously at 1.50 p. m.; 4.30 p. m., tremors observed, but no other symptoms.

October 29: Rabbit in good condition.

November 1: Weight, 950 grams; 10.55 a. m., 11 cc 2 per cent caffein injected subcutaneously (231 mg per kilo).

November 4: Weight, 930 grams; 2.50 p. m., 12 cc 2 per cent caffein injected subcutaneously (258 mg caffein per kilo).

November 6: Weight, 945 grams; 11.45 a. m., 15 cc 2 per cent caffein (313 mg per kilo) injected subcutaneously.

November 17: Rabbit still alive; weight, 890 grams.

The results of these experiments indicate that when sufficient time is allowed between two successive injections, susceptibility to caffein is not increased. The rabbit, on the contrary, seems to acquire a tolerance for the drug, for the fourth dose was 15 per cent larger than the minimum fatal dose of caffein. This is in all probability due to the better elimination of caffein and its products of decomposition and to recovery from the deleterious effects of each dose, made possible by long intervals between injections.

The results of these experiments may be briefly summed up by stating that subminimum doses of caffein given to the rabbit daily or at intervals (not too long) do not produce any symptoms such as were observed in acute caffein intoxication, namely, increased reflexes and convulsions, or increased rate of respiration, thus showing that it is not cumulative. But evidence of undoubted summation of effect was adduced to show that if the administration of subminimum doses of caffein be continued daily for a period of 11 to 18 days the result is fatal. Tolerance, however, may be acquired, although to a limited extent only, provided sufficiently long intervals between injections are allowed to give time for repair of the injury done by the drug and to develop a mechanism for its better decomposition and elimination. Furthermore, the evidence just given indicates that the elimination of subminimum doses of caffein and its products of decomposition is probably accomplished within 24 hours or thereabouts. That the elimination of larger doses is not accomplished in this interval is made probable by the following experiment:

Gray rabbit 455. Female. Diet, oats.

October 12: Weight, 1,185 grams; 3.30 p. m., 11.5 cc 2 of per cent caffein injected into the lumbar muscles; 3 p. m., reflexes increased.

October 13: 10 a. m., rabbit weighed 1,070 grams; no symptoms of caffein poisoning, reflexes normal; 10.30 a. m., 10 cc 2 per cent caffein injected into the lumbar muscles; 11.30 a. m., rabbit jumped off the table, had convulsions, and died.

EXPERIMENTS ON DOGS.

Having gained some information respecting the effects of repeated doses of caffein on rabbits, it was of interest to find out how carnivora reacted to the drug when similarly administered. A number of dogs were used for the purpose. Considerable variation in the mode of experimentation, as will appear later, was allowed.

Since the condition of the animal, its age, environment, or diet might be factors influencing toxicity, tests were made on full-grown and on young growing dogs whose food was varied. The subjects of the experiment were kept under observation for a few days to several weeks before the administration of caffein was begun, in order to determine whether or not any morbid condition existed, as well as to ascertain whether the new environment had any effect on these animals. Caffein was given chiefly by mouth, but the subcutaneous method was also employed during a portion of the experimental period in some dogs. The initial dose, which varied for different individuals, was maintained for a variable length of time. It was then progressively increased, in most cases until the death of the animal. With larger doses the intervals between successive injections were also increased.

SERIES A.

Six dogs were used in this series. Caffein was administered by mouth for periods of six days to five weeks. It was given daily or at intervals of two, and sometimes of three, days. In a few instances the drug was withheld for four or even for seven days, and its administration was resumed at the end of this time. The initial dose in these experiments varied approximately between 40 and 140 mg per kilo. The doses were then increased gradually, and thus the maximum resistance of the subject to caffein was tested. The diet consisted either exclusively of meat or largely of carbohydrates with a minimum amount of meat to give flavor to the food.

Dog 11. Female.

Diet consisted of rice, 250 grams; cane sugar, 250 grams; meat, 50 grams; cracker meal, about 100 grams. Caffein was given by mouth daily or at intervals of one day, when the dose did not exceed 1.5 grams. Before the dose was increased to 2 grams, or approximately 0.213 gram caffein per kilo, an interval of two days was allowed. Symptoms were noticed the next day. An interval of two days was therefore allowed again at the end of which the same dose was repeated. It will be remarked that there were no symptoms this time, and the general condition of the dog seemed to be good. Two grams of caffein were, therefore, given daily during the next two days without any untoward effects; the dose was then increased to 2.5 grams. Even after this enormous quantity no symptoms were observed except slight tremors. When this dose was repeated 26 hours later, it proved fatal. No albumin or sugar was found in the urine, although the dog was fed on a very liberal carbohydrate diet. The following is a complete record of the experiment.

April 20: Urine acid, no albumin, no sugar.

April 21: Urine free from sugar.

April 22: Urine free from sugar. 1 gram caffein given in the afternoon.

April 23: 9 a. m., dog was very thirsty, drank a large quantity of water, urine did not reduce Fehling's solution.

April 24: 2.30 p. m., 1 gram caffein, no sugar in urine.

April 25: 1 gram caffein administered.

April 26: Weight, 10.6 kilos, urine collected in the morning, no sugar; 4.10 p. m., 1.5 grams caffein.

April 27: 1.5 grams caffein; 1.30 p. m., diet as before, no sugar in urine.

April 28: Weight, 10.2 kilos, no caffein, no sugar in urine.

April 30: Weight, 10.4 kilos, no sugar in urine; 4.20 p. m., 2 grams caffein.

May 1: Urine examined, sugar absent, weight 10 kilos, vomited, sick, tremors observed, drank 500 cc water at one time, appetite poor.

May 2: No caffein, drank 150 cc water.

May 3: Urine, no sugar, moderate quantity of albumen present; 12 noon, 2 grams caffein given by mouth, weight 10.3 kilos; 2 p. m., urine, sugar negative, condition of dog good, no symptoms of caffein intoxication.

May 4: 10 a. m., about 10 cc thick, dark-colored mucilaginous urine found in collecting bottle; albumin a little more than a trace, decidedly less than on May 3, no sugar, condition of dog pretty good except for slight muscular tremors; 4 p. m., 2 grams caffein by mouth (as usual).

May 5: Urine not examined, no symptoms; 4 p. m., 2 grams caffein.

May 6: Urine not examined; 2.30 p. m., 2.5 grams caffein given by mouth; 4 p. m., slight tremor, no other symptoms.

May 7: No examination of urine, no symptoms observed; 4 p. m., 2.5 grams caffein.

May 8: 9 a. m., found dead, urine collected since last dose of caffein was given did not contain any sugar or albumin, the amount of caffein fed to this dog was 18 grams in 18 days. *Autopsy:* Post-mortem examination showed marked enteritis with hemorrhagic spots on the mucosa; liver and kidneys congested and dark colored; lungs congested; thyroid gland was greatly enlarged and congested.

Dog 23:

April 30: Weight, 6.8 kilos; the diet consisted of 250 grams rice, 250 grams sugar, 100 grams cracker meal, and 100 grams of meat. On May 3 his weight was 7 kilos. He received 1 gram of caffein by mouth at 12 noon. At 2 p. m. he vomited and tremors were observed. The next day, May 4, tremors were still present though less pronounced. Examination of the urine for sugar and albumin was negative; on May 4, 1 gram caffein was given again and repeated on May 5. On this date his general condition was not good—dog had no appetite and refused to take caffein. As the dog lost 10 per cent of his weight he was put on a meat diet exclusively and the dose of caffein was reduced to 0.5 gram. He became sick after the second dose, and the administration of caffein was therefore discontinued. It was resumed after five days and the caffein was administered in increasing amounts, i. e., on May 18, 0.5 gram; May 19, 0.5; May 20, 1; May 21, 1 gram in two doses of 0.5 each, given at intervals of one hour; May 22, 1 gram. Dog became irritable, but no other symptoms were observed. The administration of caffein was omitted the next day. On the following day when the same dose of caffein was given there was again marked irritability and tremors. The experiment was therefore discontinued.

Dog 22. Male bulldog.

June 24: Dog weighed 13.7 kilos. Diet consisted of meat exclusively; 1 gram caffein was given by mouth; diarrhea developed; no caffein was given for three days.

June 28: Dog weighed 13.6 kilos, 1.5 grams caffein given at 10 a. m.

June 30: 1.75 grams caffein administered.

July 2: Dog weighed 13.5 kilos; 2 grams caffein or 0.15 gram per kilo, caused well-marked thirst, but did not produce any other symptoms.

Dog 20. Female:

May 12: Weight, 7.7 kilos. Fed liberal carbohydrate diet, consisting of rice, 100 grams; sugar 100 grams; meat and cracker meal, a sufficient quantity to flavor the food.

May 14: Weight, 7.7 kilos. Examination of urine for albumin and sugar gave negative results. Urine was acid to litmus.

May 17: Weight, 7.4 kilos. Three hours after it was fed the dog received 0.5 gram caffein by mouth. The test of the urine the next day for sugar was negative, but a trace of albumin was present. It will be noticed that the doses were increased gradually and that symptoms were observed only after the fourth dose of 0.1 gram per kilo. Later meat was substituted for the carbohydrate diet and the administration of caffein was stopped for four days. At the end of this period 100 mg caffein per kilo was fed daily for five days, and the dose was then very gradually increased. Diarrhea occurred twice, but no other symptoms, the second attack having lasted a few days. The following is a complete record of the experiment:

May 19: 0.5 gram caffein 11.45 a. m.
May 20: 0.75 gram caffein 12.45 a. m.
May 21: 0.75 gram caffein 12 noon; no sugar, no albumin in urine.
May 22: 0.75 gram caffein; urine, same condition found; no symptoms.
May 23: Weight, 7.5 kilos; no caffein.
May 24: 0.75 gram caffein; tremors very marked.
May 25: No caffein.
May 26: 0.75 gram caffein.
May 27: 0.75 gram caffein.
May 28: 0.75 gram caffein.
May 29: 1 gram caffein in two doses of 0.75 and 0.25 gram.
May 30: No caffein.
May 31: No caffein; meat diet exclusively.
June 1: No caffein; meat diet exclusively.
June 2: No caffein; no sugar, no albumin in urine.
June 3: Weight, 7.6 kilos; 0.75 gram caffein; no sugar in urine.
June 4: Weight, 7.3 kilos; 0.75 gram caffein; no sugar in urine.
June 5: Weight, 7.5 kilos; 0.8 gram caffein; drank 500 cc water; ate 200 grams meat.
June 6: Weight, 7.4 kilos; 0.8 gram caffein; 500 cc urine; drank 500 cc water; ate 200 grams meat; no symptoms.
June 7: Weight, 7.7 kilos; 0.8 gram caffein 10 a. m.; 400 cc urine, 500 cc water, 200 grams meat.

June 8: Weight, 7.5 kilos; 0.9 gram caffein, 450 cc urine, 1 p. m.; 200 grams meat, 500 cc water.

June 9: Weight, 7.6 kilos; 0.9 gram caffein, 1 p. m.; 500 cc water, 200 grams meat and bone dust; diarrhea and restlessness all afternoon.

June 10: Weight, 7.6 kilos; 1 gram caffein, 500 cc water, 200 grams meat, 480 cc urine.

June 11: Weight, 8 kilos; 1 gram caffein, 470 cc urine, 500 cc water, 200 grams meat.

June 12: Weight, 7.8 kilos; 1 gram caffein, 710 cc urine, 500 cc water, 200 grams meat.

June 13: 450 cc urine, 500 cc water, 300 grams meat.

June 14: Weight, 7.9 kilos; 1.2 grams caffein, 500 cc water, 300 grams meat, 490 cc urine.

June 15: Weight 7.8 kilos, 500 cc water, 300 grams meat, 550 cc urine.

June 16: Weight 8.0 kilos, 1.2 gram caffein, 500 cc water, 300 grams meat, bone dust added to check diarrhea.

June 17: 500 cc water, 300 grams meat, 450 cc urine, diarrhea continues, bone dust added.

June 18. Weight 7.8 kilos, 1.3 gram caffein, 300 grams meat, 500 cc water, 300 cc urine.

June 19: Dog very thirsty, drank 1 liter of water and ate 350 grams of meat; 960 cc urine passed during the past 24 hour.

June 21: Weight, 7.5 kilos, 1.5 grams caffein given at 10 a. m. At 2 p. m. convulsions and death. This dog received a total of 21.15 grams caffein in 25 doses during a period of 35 days, which amounts to an average of 85 mg per kilo daily.

Dog 19. Female fox terrier.

May 13: Weight 6.4 kilos. Diet consisted of rice, 100 grams; sugar, 100 grams; and a sufficient quantity of meat and cracker meal to give flavor to the food. Examination of the urine showed a trace of albumin but no sugar. The urine was acid to litmus. Two days later the urine was alkaline to litmus. There was still a small amount of albumin but no sugar.

May 17, 0.5 gram caffein was given by mouth. Examination of the urine collected the next day still showed the presence of albumin and the absence of reducing substances. The dog had tremors. Caffein was, therefore, not administered.

May 19: 0.5 gram caffein was given by mouth.

May 20: 0.75 gram caffein was fed at 12.45 p. m. The dog vomited during the night and tremors were observed the next morning. The urine collected was examined for albumin and sugar, but neither was found.

May 21: 12 noon, 0.75 gram caffein was fed. The dog weighed 6 kilos, which therefore represented a loss of 0.4 kilo. Grew abnormally thirsty and lost appetite, but no other symptoms of caffein poisoning were observed.

May 22: The dog was again given 0.75 gram caffein at 12 noon. The examination of the urine for albumin and sugar gave negative results. The dog died at 4.15 p. m. The fatal dose for this dog was therefore 0.125 gram caffein per kilo, and the total amount of caffein ingested in six days amounted to 3.25 grams, or 0.54 gram per day, which makes 90 mg per kilo.

Dog 21. White female bull.

This dog was kept on a diet exclusively of meat, and was given water ab libitum. From 0.5 to 0.6 gram of caffein was administered daily for seven days; the doses were then increased and were given at longer intervals. No symptoms of the effects of caffein were observed until a dose of 1.5 gram was fed, when diarrhea was noticed on the next day. In the following record the details of the experiment are given:

Date.	Weight.	Caffein.	Date.	Weight.	Caffein.
	Kilos.	*Grams.*		*Kilos.*	*Grams.*
June 7	12.5	0.5	June 16	12.7	0.8
June 8	12.5	.5	June 18	12.9	1.0
June 9	12.5	.5	June 21	13.4	1.2
June 10	12.3	.6	June 24	13.3	1.5
June 11	12.3	.6	June 25	(1)	.0
June 12	12.3	.6	June 27	13.5	1.5
June 13	12.3	.6	June 30	13.5	1.75
June 14	12.3	.8			

[1] Diarrhea.

July 2: 11.30 a. m., 2.0 gram caffein fed by mouth; 1.30.p. m., tetanus, dog died. The total amount of caffein fed to dog No. 21 out of the 25 days of the experiment was 14.45 grams, or an average of 578 mg per day, which amounts to about 42 to 43 mg per kilo of body weight.

Notwithstanding the diversity in the method of experimentation, there was a striking uniformity in some of the results obtained. All the experiments of the series showed absence of cumulative action of caffein. The experimental evidence presented indicates that moderately large doses may be given at intervals of about 24 hours without inducing any symptoms of nervous or any other disturbance. This is illustrated in the tests on dog 11, which were preliminary in character. In this subject 100 to 150 mg of caffein per kilo were ingested daily for several days without showing any changes. Later in the course of the experiment, after larger doses were given, mild symptoms only, such as tremors, were observed. Additional evidence of the absence of cumulative action of caffein was furnished by the results of the following experiments:

Dog 23 received 142 mg of caffein per kilo on three successive days. His general condition indicated that these amounts of caffein were toxic, but he survived. In another series of tests, made after he was allowed to rest a few days, he again failed to show any cumulation of the drug, as he survived this time a series of tests of longer duration than the first.

A much better illustration of the absence of cumulative action of the drug is furnished by the experiments on dog No. 20. In this case 100 to 125 mg of caffein per kilo, given on 10 consecutive days, did not cause any marked effects. Diarrhea and restlessness were the only symptoms observed. These experiments therefore show that the elimination and decomposition of caffein are apparently effected by the body within twenty-four hours or thereabouts.

Experiments on dog 19, however, form an exception—the third dose of 125 mg caffein per kilo having proved fatal. The very low protein content of the diet of this dog suggests itself as a possible cause of the lower resistance to caffein of this subject. But it may be observed that the same diet was furnished to dog 20, which stood such amounts of caffein much longer. The presence of a trace of albumin in the urine of dog 19 is likewise inadmissible as a cause of the difference in the toxicity of caffein in this dog, for the urine of dog 20 likewise contained a trace of albumin. The alkaline reaction of the urine, together with the fact that the first dose of only 60 mg of caffein per kilo induced symptoms of toxicity, suggests the presence of an abnormal condition which in all probability was the cause of the death of this subject under the conditions indicated.

In a large number of experiments on caffein performed in this laboratory it has been observed that symptoms due to caffein often disappeared when the administration of the same dose of the drug was continued. Thus dog 19 vomited when the amount of caffein was increased to 125 mg per kilo. When this amount was repeated the next day there was no vomiting. Similar observations were made on dogs 11 and 23, also on other dogs. No. 22 developed diarrhea at first; when the administration of caffein was resumed several days later, however, there was no diarrhea. In other experiments performed in this laboratory, symptoms of nervous irritability induced by caffein disappeared on continued treatment.

It was interesting, therefore, to inquire whether resistance to caffein would be increased by the continued administration of progressively larger amounts of the drug. When doses of 150 and over were fed, the intervals allowed were usually longer than 24 hours. Two and sometimes three days were permitted to elapse between two successive doses. This was done in order to allow time for recovery from possible changes induced by larger doses of caffein, and thus prevent the summation of effect. In the experiments considered, therefore, Nos. 11, 23, 20, and 19, the toxicity of caffein does not seem to be greater than in the experiments on acute caffein intoxication in the dog. It was thought, however, that the large initial doses or the quick change to large doses when the amounts used in the beginning were small, might have something to do with failure to induce a marked degree of tolerance. The experiment on dog 21 was therefore carried out by giving from 40 to 60 mg per kilo for eight days, and then increasing the dose, but tolerance could not be induced, as is shown in the protocol to the experiment.

SERIES B.

According to the studies of Chittenden,[16] low protein diet improves the general metabolism of the body, fatigue is diminished, and bodily vigor, therefore, correspondingly increased. The expectation is, therefore, justified that the defense of the organism against deleterious substances introduced into the body is much improved by such a diet, thus increasing its resistance to poisons. Hunt's experiment on this subject, also quoted by Chittenden, lends support to this view. He found that mice fed on carbohydrates chiefly, or on foods containing only a small amount of protein, were more resistant to acetonitril. It was interesting, therefore, to inquire whether the toxicity of caffein differs under similar conditions of diet.

A fixed diet of the same calorific value was provided for all dogs of this series, but the protein content for three of the animals was approximately one-third of the amount usually fed to dogs. Caffein was at first administered subcutaneously, but all the dogs on a low protein diet developed abscesses at the site of injection, while none of those on high protein diet showed a local reaction. Feeding by mouth was then begun and continued throughout the experiment in each case. The initial dose was 50 mg per kilo, which was given daily for seven to nine days. It was then increased progressively by 25 mg per kilo; 75 mg per kilo were administered for one to two days, 100 mg for two to three days, 125 mg for one to two days, 150 mg for one to two days, and a single dose of 175 mg. It will be remarked that sometimes an interval of one day had to be allowed during which no caffein was fed.

Dog 30. Black and tan hound, male.

The dog was under observation for about eight weeks before the experiment was begun and had received a high protein diet. He was then given 50 mg caffein for nine consecutive days. On the tenth day the dose was increased to 75 mg per kilo. As no symptoms developed, this dose was increased to 100 mg per kilo, and was fed one day apart. It was then raised to 125 mg per kilo. For the first time since the drug was fed, symptoms appeared; they were noticed a few hours after feeding and persisted during the next day. Although the appetite was good, no caffein was given on this day. On the following day this dose was repeated. As the symptoms were not serious, 150 mg per kilo were given daily for the next three days, until 175 mg per kilo was reached. This dose proved fatal within six hours. Record of experiment follows:

October 9: Weight, 9 kilos, on full nitrogen diet, received daily 0.724 gram nitrogen per kilo or 87 calories per kilo, received 18 grams meat per kilo, 4 grams lard per kilo, 3 grams carbohydrates per kilo, bone dust, ad libitum.

November 3: Weight, 9.10 kilos.

November 10: Weight, 9 kilos.

November 20: Weight, 9.55 kilos.

November 29: Weight, 8.70 kilos.

December 6, 7, 8, and 9: Received subcutaneously 22 cc 2 per cent caffein. Condition good, site of injection normal.

December 10, 11, 12, 13, and 14: Received 0.4375 gram caffein by mouth equal to 0.050 gram per kilo, no symptoms, appetite and general condition good.

December 15: 11.30 a. m., received 0.6563 gram caffein by mouth, or 0.75 gram per kilo, no symptoms, appetite good, condition excellent.

December 16: 11 a. m., received 0.870 gram caffein by mouth, or 0.1 gram per kilo, weight 8.70 kilos, no symptoms.

December 17: No caffein given.

December 18: Received 0.870 gram caffein, or 0.1 gram per kilo, no symptoms.

December 20: 2.45 p. m., received 1.0875 grams caffein, or 0.125 per kilo; 4 p. m., ate food readily, seemed very uncomfortable and sick.

December 21: 9 a. m., stiffness in muscles, but no other symptoms, appetite good, no caffein given.

December 22: 11 a. m., received 1.0875 grams caffein, or 0.125 gram per kilo; 3 p. m., depressed in spirits and sick, but no other symptoms observed.

December 23: 11.30 a. m., received 1.305 grams caffein, or 0.150 gram caffein per kilo; 1.30 p. m., apparently quite sick, but no other symptoms, had good appetite.

December 24: 10 a. m., received 0.175 gram caffein per kilo; 4 p. m., when about to be fed fell over and died; no autopsy.

The total amount of caffein given dog 30 was 11.3458 grams, administered for a period of eighteen days. The average daily amount per kilo was therefore 72 mg. The feces became offensive when the amounts of caffein were increased to 75 mg per kilo. It will be observed that in this dog the appetite was uniformly good until the day of his death. Whether or not this is the cause of his resistance to caffein will be discussed later.

Dog 32. White, male, young.

Although he was growing rapidly this dog's weight was constant, but he looked anemic. He received a high protein diet until December 3, when the rations were increased by one-third. This dog was under observation from October 26 to December 6 when the administration of caffein was begun. He then received 50 mg caffein per kilo daily for nine days consecutively without showing any effects, when the dose was increased to 75 mg per kilo, then to 100 mg per kilo. This dose was further increased to 150 mg per kilo without causing symptoms, which was repeated the next day. No symptoms having been observed after such amounts of caffein, 175 mg per kilo were fed. This dose, however, proved fatal within two hours. Record of experiment follows:

October 26: Weight, 6.90 kilos.
November 3: Weight, 6.90 kilos.
November 10: Weight, 6.90 kilos.
November 20: Weight, 6.90 kilos.
November 29: Weight, 6.55 kilos.
December 3: Put into cage, diet increased one-third.

December 6, 7, 8, 9: Weight 6.30 kilos; 12.30 p. m., received 16 cc 2 per cent caffein by subcutaneous injection in back, no symptoms of any kind noticed, site of injection normal.

December 10, 14: 0.05 gram caffein per kilo.
December 15: Received 0.4725 gram caffein by mouth, no symptoms.
December 16: Received 0.655 gram caffein, 0.100 gram per kilo.
December 17: No caffein given.
December 18: Received 0.655 gram caffein daily, 0.100 gram per kilo, no symptoms.

December 20: Received 0.8188 gram caffein, 0.125 gram per kilo, no symptoms, appetite good.

December 21: Received 0.9825 gram caffein, 0.150 gram per kilo, somewhat uncomfortable, no other symptoms.

December 22: Received 0.9825 gram caffein, 0.150 gram per kilo, no symptoms except some uneasiness.

December 23: 9 a. m., no symptoms, appetite good; 11.30 a. m., received 1.146 grams caffein, 0.1759 gram per kilo; 1.30 p. m., died while making an effort to get out of cage, tonic contraction of limbs observed before death.

The amount of caffein received during the entire experimental period was 9.2223 grams, or an average per day approximately of 80 mg per kilo, and therefore 10 per cent more than dog No. 30 received. It will be observed that the appetite in dog No. 32 was likewise uniformly good, and that he received a very high protein diet which was also of a very high calorific value.

Autopsy (*dog 32*).—Stomach presented a severe inflammation of the mucosa, especially in the fundus and pyloric portions. The gastritis was more marked in pyloric portion, and the inflammatory condition extended along the whole course of small intestines, which presented numerous hemorrhagic areas, and a thick catarrhal exudate on the mucosa. The large intestine contained quite a large number of parasites, probably round worms. The liver was enlarged and the gall cyst well filled. The spleen was also considerably engorged, kidneys appeared normal, other organs all appeared normal.

Dog 31. Black spaniel, male.

This dog had been under observation one month previous to the experiments with caffein. The usual initial dose was then administered for nine days. There were no signs of local irritation when the drug was given subcutaneously, but symptoms of toxicity were present. These disappeared, however, when the drug was administered by mouth. The dose was therefore increased to 75 mg per kilo. This, as will be seen, proved fatal within six hours. High nitrogen diet, same as No. 30.

November 3: Weight 10.250 kilos.

November 10: Weight, 10.25 kilos.

November 20: Weight, 10.30 kilos.

December 1: Put in cage.

December 6, 7, 8, 9: Weight, 10.20 kilos; received 26 cc 2 per cent caffein subcutaneously, site of injection normal.

December 6: Very restless and excited, whined when handled as though muscles were sore, appeared to be sick.

December 10–14: Condition good, received 0.51 gram caffein by mouth daily, no noteworthy symptoms, appetite continues good, somewhat restless at intervals.

December 15: 11.30 a. m., received 0.765 gram caffein per mouth (0.075 gram per kilo); 2 p. m., depressed in spirit, seemed sick and uncomfortable; 4.15 p. m., when about to feed, animal jumped up, then fell back dead.

Autopsy (*dog 31*): Lungs congested; heart filled with blood and contained small amount of blood-stained fluid in pericardial sac. Liver deeply congested, soft and friable; gall bladder distended with bile; kidneys showed inflammation of cortex; spleen pale, normal in size and consistency; stomach practically empty, the mucosa of the pyloric portion exhibited severe gastritis, with thick catarrhal exudate. This catarrhal inflammation extended through the duodenum; remaining portion of small intestine showed mild inflammation; large intestine appeared practically normal. The total amount of caffein received by dog 31 during 10 days was 5.395 mg, or a daily average of 53.9 mg per kilo. This unusually low resistance to caffein (which was practically the only case in all the experiments on dogs presented in this research) suggests the presence of some abnormal condition. The bloody exudate in the pericardial cavity indicating pericarditis, which is likely to induce secondary changes of cardiac muscle, may be considered as a possible cause of the increased toxicity of caffein in this case.

Dog 29. Male fox terrier, black.

This dog was kept on a low nitrogen diet for nearly five weeks before the feeding of caffein was begun. The administration of 50 mg of caffein per kilo was then carried on for eight days without showing any symptoms of toxicity. The usual increase of dose was then given—75 mg per kilo—which was followed by a manifestation of symptoms. Further increase, however, to 100 mg per kilo had no visible effect. Nevertheless it was considered advisable to suspend the feeding of caffein for one day. The same amounts were then repeated on two consecutive days. No symptoms having been observed, 125 mg per kilo were given. As symptoms of toxicity and especially loss of appetite were observed, the dog was not given any caffein the next day. Since his appetite had now improved, the experiment with larger doses was resumed. Death followed after the second dose of 150 mg per kilo. Protocol follows:

Weight, 9.90 kilos. One-third nitrogen diet. Receives 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 9.85 kilos.

November 10: Weight, 9.55 kilos.

November 12: Weight, 9.40 kilos.

November 29: Weight, 9.85 kilos.

December 6: Weight, 9.90 kilos; 11.35 a. m., received 25 cc 2 per cent caffein solution by subcutaneous injection in back; 4 p. m., no symptoms, appetite good.

December 7–9: Received 25 cc caffein 2 per cent solution—subcutaneous injection, no symptoms, area of injection inflamed and swollen.

December 10, 13: Site of injection showed increased inflammation, received 0.495 gram caffein (50 mg per kilo) in 30 grams meat daily without showing any symptoms.

December 14: 12 noon, received 0.7425 gram caffein by mouth (0.075 per kilo); 2.30 p. m., restless and uneasy.

December 15: 11.30 a. m., received 0.7425 gram caffein by mouth; 2 p. m., depressed in spirits, although continues to have good appetite.

December 16: Weight, 9.50 kilos; 3.15 p. m., received 0.9509 gram caffein by mouth; 4.50 p. m., no symptoms.

December 17: Animal rested.

December 18: Received 0.950 gram caffein by mouth, no symptoms.

December 19: Received 0.9509 gram caffein by mouth, no symptoms.

December 20: 2.45 p. m., received 1.1875 grams caffein (0.125 gram per kilo); 4 p. m., restless and quite sick; ate only a little food.

December 21: 9 a. m., still uncomfortable, allowed to rest, no caffein given, gradually recovered appetite.

December 22: 11 a. m., received 1.875 grams caffein; 3 p. m., seemed sick, but showed no other symptoms, appetite fair.

December 23: 9 a. m., showed no symptoms from the day before, ate food gradually, seemed sick; 11.30 a. m., received 1.425 grams caffein (0.150 gram per kilo); 1.30

p. m., looked and behaved as if very sick, no other symptoms; 3.45 p. m., in attempting to get out of box fell over on back, had convulsions, whined, dyspnœa, died within 30 seconds.

Autopsy: Stomach exhibited mild inflammation; small intestine inflamed and hemorrhagic areas on mucosa; liver engorged and friable; spleen normal; kidneys slightly congested; other organs appeared normal. The total amount of caffein fed to Dog 29 was 12.135 grams, which was given in 18 days. The average daily amount per kilo was therefore 67.68 mg.

Dog 28. Male fox terrier.

Low nitrogen diet was begun about four weeks before the feeding of caffein; 50 mg of caffein was then fed for seven consecutive days. Partial loss of appetite was observed after the first dose. As the experiment progressed the desire for food steadily diminished, and the feces became fetid. Symptoms of intoxication manifested themselves early in the experiment, and vomiting occurred after the fourth dose. The dog was then put on a diet exclusively of meat. After an intermission of 10 days 109 mg caffein per kilo were given. Since there were no symptoms, the following day the amount was increased to 125 mg per kilo. This dose proved fatal within 16 to 20 hours.

This dog was stout and strong, weight 12.25 kilos, received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

November 3: Weight, 11.75 kilos.

November 10: Weight, 11.95 kilos.

November 20: Weight, 11.20 kilos. All through this period had been kept in a cold, poorly ventilated room, put in a warm room, with bedding and good ventilation.

November 29: Weight, 11.95 kilos.

December 1: Put in a cage; weight, 11.95 kilos.

December 6: Weight, 11.95 kilos; 11.45 a. m., received 0.050 gram caffein per kilo; then received 30 cc 2 per cent caffein (0.6 gram) in practically one subcutaneous injection; 4.30 p. m., ate only part of food.

December 7: 10.25 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (0.6 gram, or 50 mg, per kilo); 1.45 p. m., seemed sensitive to touch, no desire for food, depressed in spirit.

December 8: 11.40 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 1 p. m., depressed in spirit, hind legs seemed somewhat stiff, no desire for regular food, site of injection inflamed.

December 9: 10.50 a. m., received 30 cc 2 per cent caffein by subcutaneous injection (50 mg per kilo); 2.30 p. m., had vomited, no desire for regular food.

December 10: Inflammation of site of injection, and swelling very pronounced; 2 p. m., received 0.5975 gram caffein, or 50 mg per kilo, with 30 grams of meat, refused regular food.

December 11, 12: Received 0.5975 gram caffein by mouth, no symptoms except refusal of regular food, feces fetid.

December 13–22: Put on meat diet exclusively, high temperature, no caffein, weight 10 kilos, appetite good, feces fetid.

December 22: 12 m., weight 11 kilos, received 1.2 grams caffein by mouth (0.109 gram per kilo); 4 p. m., no symptoms.

December 23: 11.30 a. m., received 1.375 grams caffein (0.125 gram per kilo) had vomited food of the day before, but could notice no caffein or capsules in vomit; 4.30 p. m., no symptoms, seemed in good spirits, appetite good, had no meat to feed with, so was given low nitrogen feed, of which he ate about one-fourth.

December 24: 9 a. m., found dead, stiff, and cold. The most striking effect of caffein in this dog is the increased intestinal putrefaction. The feces were still fetid 10 days after the administration of caffein was stopped.

Autopsy, dog 28: Stomach partially filled with an undigested food mass; mucosa showed severe inflammation; small intestines presented a hemorrhagic enteritis along whole extent; large intestine also exhibited mild inflammation; liver was engorged; spleen appeared normal; kidneys slightly congested in cortical portion; other organs appeared normal.

Dog 24. White and tan male: Was put on low protein diet six weeks before experiments with caffein were begun. The initial dose of 50 mg per kilo was then administered on eight consecutive days. The only symptoms observed during this period of caffein administration were those of intestinal putrefaction. Fetid feces were noticed already after the first dose of caffein was injected. When the second dose of 75 mg of caffein was repeated, mild symptoms appeared, but none have been observed even with increased amounts of caffein.

One-third nitrogen diet. Received daily 0.269 gram nitrogen per kilo (88.269 calories per kilo).

October 26: Weight 11.15 kilos. Food consisted of 5 grams cracker meal per kilo; meat, 3 grams per kilo; lard, 2 grams per kilo; tapioca, 10.69 grams per kilo. Kept in a cold, damp room with poor ventilation until November 20.

November 3: Weight, 11 kilos.

November 10: Weight, 10.75 kilos.

November 20: Weight, 10.55 kilos; changed to a warm room, with bedding and good ventilation.

November 29: Weight, 10.85 kilos.

December 1: Put into a cage.

December 6: Weight, 10.90 kilos; 11.25 a. m., received 28 cc 2 per cent caffein subcutaneously in side, below the shoulders, area washed with alcohol and ether, approximately 50 mg per kilo administered, no symptoms.

December 7: 10.15 a. m., received 28 cc 2 per cent caffein injected subcutaneously; feces soft and very fetid; 1 p. m., depressed in spirit, eyes dull.

December 9: 10.45 a. m., received 25 cc 2 per cent caffein solution subcutaneously, feces still fetid, site of injection inflamed and swollen, no other symptoms.

December 10: Inflammation of area of injection more pronounced; 2 p. m., given 0.5449 gram caffein and 30 grams of meat; 4 p. m., fed, no symptoms, feces fetid.

December 11: 12 m., given 0.5459 gram caffein and 30 grams of meat, no symptoms, feces fetid.

December 12, 13: Given 0.5459 gram caffein daily, without noticing any symptoms.

December 14: 12 m., received 0.817 gram caffein (75 mg per kilo); 2.30 p. m., restless and uncomfortable, no other symptoms.

December 15: 11.30 a. m., received 0.8175 gram caffein by mouth; 2 p. m., depressed in spirit, acted as though sick, no other symptoms.

December 16: Weight, 11 kilos; 11 a. m., received 0.100 gram caffein per kilo (1.100 grams) by mouth, no symptoms.

December 17: Rested.

December 18: 2.30 p. m., received 1.100 grams caffein by mouth; 4. p. m., no symptoms.

December 19: 12 noon, received 1.100 grams caffein by mouth; 4.15 p. m., no symptoms.

December 20: 2.45 p. m., given 1.375 grams caffein (0.125 gram per kilo); 3.45 p. m., vomited—one of the capsules being found intact, the other broken open; 4 p. m., given regular diet, containing 1.3757 grams caffein in capsules, ate most of this during the night, whined at intervals, coordination disturbed, appeared very sick, but exhibited no other symptoms.

December 21: 9 a. m., found dead, stiff, and cold.

The total amount of caffein received by dog 24 was between 10.109 and 11.484 grams. As one of the capsules vomited was intact and the other broken open, the amount was probably about 10.75 grams. The fatal dose in this case was undoubtedly less than 185 mg per kilo—somewhere between 125 and 185 mg. Autopsy showed heart in diastole; posterior lobe of right lung deeply congested; liver engorged; gall cyst filled; spleen appeared normal; stomach well filled with semifluid mass; pyloric portion of stomach exhibited a severe inflammation of mucosa; mucosa of duodenum greatly inflamed and showed hemorrhagic areas and catarrhal exudate; remainder of small intestine also exhibited mild inflammation; kidneys deeply engorged, mesentery injected.

A comparison of the fatal doses of caffein in the experiments on high and low protein diet does not show much difference in the resistance to caffein, since 175 mg per kilo proved fatal to Nos. 30 and 32, while No. 29 died after receiving 150 mg per kilo, and No. 24 received 125 to 185 mg per kilo. Moreover, No. 28, which was changed from low to high protein diet, succumbed when given 125 mg per kilo. Observations made during the experimental period indicate, however, greater toxicity of caffein in the subjects on low protein diet. Dog 30 showed the effects of the drug when the dose was increased to 125 mg of caffein per kilo, while in No. 32, 150 mg per kilo were received without any manifestation of symptoms. Dog 31, which was likewise on a high protein diet, is evidently an exception, and its low resistance to caffein may be accounted for by the condition found at autopsy. In other dogs on low protein diet symptoms of intoxication appeared early in the experiment. In Nos. 29 and 24 it was observed as soon as the amount of caffein was increased to 75 mg per kilo. In dog 28 the first dose of caffein 50 mg per kilo was toxic. The symptoms of gastro-intestinal disturbance were especially marked after caffein on low protein diet. This may seem to contradict the results of experiments on dogs 11 and 20, in which larger doses of caffein failed to induce symptoms of intoxication. But it should be observed that the diet, which consisted almost exclusively of carbohydrates, was given only during the administration of caffein, while in the experiments of series B the subjects received a low protein diet for several weeks before the administration of caffein was begun, and it was continued through the entire caffein period. It will be remarked that the absence of cumulative action in the experiments of the preceding series was also observed in dogs on high as well as on low protein diet. The appearance of symptoms after smaller doses of caffein in the latter experiments might suggest cumulative action, but since these symptoms disappeared on continued administration of the substance cumulation is clearly not indicated. The gastrointestinal lesions observed on post-mortem examination were, it will be recalled, also found in rabbits similarly treated. The explanation suggested probably applies also in the case of dogs.

SERIES C.

As already pointed out in the experiments on acute toxicity of caffein, young growing dogs are probably more resistant to caffein than adults. That this may also hold true in chronic caffein intoxication seemed indicated by the following experiments.

Dog 33. Black female puppy. Weight, 4 kilos. Had been continuously on a meat diet.

December 22: 2.30 p. m., received 0.69 gram of caffein (0.172 gram per kilo); 3.15 p. m., no symptoms except that feces were fetid.

December 23: 11.30 a. m., received 0.79 gram of caffein (0.197 gram per kilo); 1.30 p. m., no symptoms.

December 24: 11 a. m., received 0.87 gram of caffein (0.2009 gram per kilo); 4 p. m., no symptoms.

It will be observed that the only effect produced in dog 33 by feeding caffein was increased intestinal putrefaction, although 2.37 grams of caffein were given in three days. Additional data on the effects of the age of animals on the resistance to caffein seemed desirable. The following experiments were therefore carried out. Six puppies of the same litter were weaned when 7 to 8 weeks old and put on a milk diet. Three of them received this diet throughout the experimental period. Meat was substituted in the other three a few days before the administration of caffein was begun, and was continued until the end of the experiment. Caffein was given by mouth; the initial dose, which was administered for several days and then gradually increased, being 160 to 200 mg for each dog, except one, which received only 100 mg per kilo for several days and then an increased amount.

An intermission of a few days (during which no caffein was given) was allowed. This was done on account of some studies carried on at the same time on the effect of caffein on certain constituents of the urine.

PUP NO. 1.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,450	300	10.0	No symptoms.
Apr. 22	1,520	300	10.0	Do.
Apr. 23	1,450	250	10.0	Do.
Apr. 24	1,375	250	10.0	Do.
Apr. 25	1,420	250	10.0	Do.
Apr. 26	1,390	250	None.	
Apr. 27	1,400	250	None.	
Apr. 28	1,405	250	None.	
Apr. 29	1,420	250	None.	Passed worms.
Apr. 30	1,430	250	None.	Do.
May 1	1,450	250	10.0	No symptoms.
May 2	1,515	250	15.0	Do.
May 3	1,475	250	15.0	Do.
May 4	1,495	250	15.0	Do.
May 5	1,515	250	22.0	Seems dull and whines.
May 6	1,535	250	20.0	Whines.
May 7	1,525	250	20.0	No symptoms.
May 8	1,530	250	20.0	Do.
May 9	1,500	250	23.0	Diarrhea; passed worms; tremor and rigidity of legs; whines.
May 10	1,490	250	None.	Completely recovered from the effects of 9th.
May 11	1,535	250	25.0	Can not balance itself; continually vomiting.
May 12	1,460	300	None.	Recovered from effects.
May 13	1,475	350	None.	In good condition.
May 14	1,545	250	None.	
May 15	1,550	250	None.	
May 16	1,555	250	None.	
May 17	1,560	250	25.0	Salivated in cage; stiffness of muscles.
May 18	1,450	250	None.	Weak and stiff; diarrhea.
May 19	1,500	250	None.	No symptoms.
May 20	1,565	250	None.	
May 21	1,545	250	None.	
May 22	[1]	250	None.	
May 23	1,595	250	27.0	Tremors; gait clumsy; incoordination of movements.
May 24	1,495	250	27.0	Diarrhea; vomited; weak and stiff; found dead 9 a. m. 25th.

[1] Sunday.

Autopsy: Marked pulmonary congestion; liver very pale; heart wall injected; slight inflammation of stomach and intestines.

PUP NO. 2.

Date.	Weight.	Food (milk).	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*cc.*	*cc.*	
Apr. 21	1,350	300	5.0	No symptoms.
Apr. 22	1,240	300	5.0	Do.
Apr. 23	1,250	200	7.5	Do.
Apr. 24	1,205	200	7.0	Do.
Apr. 25	1,220	200	7.0	Do.
Apr. 26	1,210	200	None.	
Apr. 27	1,210	200	None.	
Apr. 28	1,205	200	None.	
Apr. 29	1,200	200	None.	Passed worms.
Apr. 30	1,210	200	None.	
May 1	1,220	200	10.0	No symptoms.
May 2	1,220	200	10.0	Do.
May 3	1,235	200	10.0	Do.
May 4	1,235	200	10.0	Do.
May 5	1,235	200	17.0	Whines.
May 6	1,250	200	17.0	Do.
May 7	1,235	200	15.0	Diarrhea and worms.
May 8	1,250	200	15.0	Diarrhea.
May 9	1,165	200	18.0	Little or no symptoms.
May 10	1,235	200	None.	No symptoms.
May 11	1,300	200	20.0	Salivated in cage; refused to eat; draws up hind legs.
May 12	1,200	200	None.	Recovered.
May 13	1,215	200	None.	In good condition.
May 14	1,280	200	None.	
May 15	1,300	200	None.	
May 16	1,310	200	None.	
May 17	1,310	200	20.0	Salivated in cage; stiff; all symptoms.
May 18	1,250	200	None.	Weak and stiff.
May 19	1,245	200	None.	No symptoms.
May 20	1,310	200	None.	
May 21	1,325	200	None.	
May 22	1,325	200	None.	
May 23	1,325	200	22.0	Somewhat stiff.
May 24	1,315	200	22.0	Restless; scratches eyes; sick.

PUP NO. 3.

Apr. 21	1,215	300	None.	
Apr. 22	1,220	300	None.	
Apr. 23	1,220	200	None.	
Apr. 24	1,200	200	None.	
Apr. 25	1,205	200	None.	
Apr. 26	1,195	200	None.	
Apr. 27	1,200	200	(1)	
Apr. 28	1,215	200	None.	
Apr. 29	1,220	200	None.	
Apr. 30	1,200	200	None.	
May 1	1,225	200	10.0	No symptoms.
May 2	1,230	200	10.0	Do.
May 3	1,235	200	10.0	Coughs and whines.
May 4	1,245	200	10.0	Passed worms.
May 5	1,270	200	17.0	Eyes appear dim and is continually scratching them.
May 6	1,260	200	17.0	Appears restless and draws up hind legs when walking.
May 7	1,240	200	15.0	Eyes dim; passed worms; diarrhea.
May 8	1,205	200	15.0	Coughing continually; very restless.
May 9	1,240	200	18.0	12 noon; salivated in cage; passed worms; diarrhea; foaming at mouth; can not balance himself; rigidity and tremor of hind legs. 2.15, found dead.

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(1) Urine squeezed from bladder.

Autopsy: Severe pulmonary congestion; catarrhal gastritis; mild enteritis with small hemorrhagic areas on mucosa.

PUP NO. 4.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	cc.	
Apr. 28	1,670	300	....	
Apr. 29	1,670	300	....	
Apr. 30	1,670	300	....	
May 1	1,690	300	....	
May 2	1,690	300	....	
May 3	....	300	....	
May 4	1,720	300	....	
May 5	1,735	300	....	
		Meat (*grams*).		
May 6	1,760	60	....	
May 7	1,745	80	....	
May 8	1,710	180	....	
May 9	1,750	180	....	
May 10	1,750	180	....	
May 11	1,755	180	10.0	No symptoms.
May 12	1,730	180	None.	
May 13	1,785	180	None.	
May 14	1,835	115	10.0	Do.
May 15	1,820	115	10.0	Do.
May 16	1,835	115	10.0	Passed worms.
May 17	1,860	115	10.0	Feces soft and black.
May 18	1,855	115	15.0	Stiff; loss of appetite.
May 19	1,770	115	15.0	Loss of appetite.
May 20	1,755	115	15.0	Do.
May 21	1,780	115	17.0	Restless.
May 22	....	115	17.0	Feces soft and black.
May 23	1,785	115	17.0	Loss of appetite.
May 24	1,795	115	....	Loss of appetite; threw up worms.
May 25	1,630	115	20.0	Loss of appetite; worms; cough; diarrhea.
May 26	1,600	115	23.0	Weak; no appetite; diarrhea; cough.
May 27	....	....	....	Found dead, 9 a. m.

Autopsy.—Lung uniformly congested; liver deeply congested; heart muscle pale with hemorrhagic areas; kidneys pale with hemorrhagic spots on surface and in cortex; slight catarrhal inflammation of stomach and the small intestines.

PUP NO. 5.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	*cc.*	
Apr. 28	1,745	300	....	
Apr. 29	1,745	300	....	
Apr. 30	1,750	300	....	
May 1	1,765	300	....	
May 2	1,765	300	....	
May 3	....	300	....	
May 4	1,490	300	....	
May 5	1,805	300	....	
		Meat (*grams*).		
May 6	1,815	60	....	
May 7	1,825	80	....	
May 8	1,770	180	....	
May 9	1,795	180	....	
May 10	1,805	180	....	
May 11	1,800	180	10.0	No symptoms.
May 12	1,720	180	None.	
May 13	1,815	180	None.	
May 14	1,845	115	10.0	Do.
May 15	1,830	115	10.0	Do.
May 16	1,815	115	10.0	Loss of weight; no other symptoms.
May 17	1,830	115	15.0	No symptoms.
May 18	1,835	115	15.0	Stiffness.
May 19	1,825	115	15.0	No symptoms.
May 20	1,850	115	15.0	A little stiff.
May 21	1,835	115	17.0	No symptoms.
May 22	....	115	17.0	
May 23	1,820	115	17.0	Do.
May 24	1,835	115	20.0	Do.
May 25	1,840	115	20.0	Feces soft and black.
May 26	1,820	115	23.0	
May 27	1,840	115	25.0	A little stiff.
May 28	1,830	115	25.0	
May 29	....	115	None.	
May 30	....	115	None.	
May 31	1,770	115	None.	
June 1	1,765	115	25.0	Diarrhea; stiff in hind legs.
June 2	1,750	115	27.5	Diarrhea and worms.
June 3	1,635	....	27.5	Paralyzed; vomited; died at 3 p. m.

PUP NO. 6.

Date.	Weight.	Food.	Treatment (2 per cent caffein).	Symptoms.
	Grams.	*Milk* (cc).	cc.	
Apr. 28	....	300	....	
Apr. 29	1,280	300	....	
Apr. 30	1,290	300	....	
May 1	1,315	300	....	
May 2	1,330	300	....	
May 3	....	300	....	
May 4	1,360	300	....	
May 5	1,365	300	....	
		Meat (*grams*).		
May 6	1,395	60	....	
May 7	1,365	80	....	
May 8	1,340	180	....	
May 9	1,380	180	....	
May 10	1,400	180	....	
May 11	1,425	180	14.5	No symptoms.
May 12	1,470	180	None.	
May 13	1,485	180	None.	
May 14	1,510	115	14.5	Do.
May 15	1,500	115	14.5	Do.
May 16	1,485	115	14.5	Passed worms.
May 17	1,480	115	14.5	
May 18	1,485	115	19.5	Feces soft and black; almost diarrhea.
May 19	1,495	115	19.5	
May 20	1,500	115	19.5	Scratches her eyes and chases her tail.
May 21	1,500	115	17.0	
May 22	....	115	17.0	
May 23	1,470	115	17.0	
May 24	1,465	115	20.0	
May 25	1,450	115	20.0	Feces soft and black.
May 26	1,450	115	23.0	Diarrhea and worms.
May 27	1,355	115	23.0	Refused to eat all food.
May 28	1,270	115	23.0	Threw up worms, stiff, and has skin over both eyes.
May 29	....	....	....	Found dead.

Highest amount of caffein given, 362 mg per kilo. No autopsy.

Examination of the results obtained in the experiments of series C shows that young and growing dogs tolerate large amounts of caffein. In four subjects of this series, Nos. 1, 2, 3, and 6, no effect was observed when moderately large amounts (160 to 200 mg per kilo of caffein) were fed. Symptoms were noticed only when these amounts of caffein were increased from 50 to 60 per cent. The other two dogs, Nos. 4 and 5, of this series were less resistant, however, to caffein, as 0.16 gram of the drug per kilo induced well-marked symptoms. Since these were fed meat, while Nos. 1, 2, and 3 received milk, the difference in toxicity may be due to the diet employed, but No. 6, which likewise received a meat diet, failed to show the effects of caffein when 200 mg per kilo were fed. On the other hand, it should be noticed that No. 1 died after receiving 360 mg per kilo, No. 2 survived a dose of 334 mg, while No. 3 died after a dose of 322 mg per kilo of caffein. The fatal doses for Nos. 4, 5, and 6 were 287, 335, and 300 mg per kilo, respectively. Although the differences are too small to justify any definite conclusion regarding the effect of a milk diet or of a meat diet on the toxicity of caffein, the results nevertheless suggest a reasonable possibility that caffein is more toxic to young dogs when on an exclusively meat diet than when fed milk. It is perfectly evident, however, that the resistance to caffein in either case is very great, almost twice that of adult subjects. As shown in series A and B, 125 to 175 mg per kilo proved fatal to all but two animals in these experiments, while symptoms of toxicity appeared after much smaller doses. In other respects the behavior of young dogs toward caffein was the same as that of the adult. In neither case was cumulation nor tolerance observed under the conditions of these experiments. The findings at autopsy were likewise similar, as gastro-enteritis was the chief lesion observed on macroscopic examination. It might be mentioned, however, in this connection, that the symptoms of caffein intoxication in young dogs often presented marked differences from those observed in those of more advanced age. The resemblance of the effects of caffein in young puppies and in rabbits was very striking. In both, the tonic with clonic convulsions were observed after a sufficient quantity of caffein was administered. In the dogs which were fully grown a large dose of caffein was usually followed by tonic convulsions and almost instantaneous death.

Moderately large amounts of caffein fed daily to puppies for several days—in some cases as long as 10 days—induced mild symptoms only. No cumulative effect was observed in any of the experiments of series C. There seems to be tolerance of certain functions toward caffein, but no general tolerance of the body could be obtained in these experiments. Caffein is apparently less toxic for adult dogs on high than on low protein diet. In young and growing dogs caffein is somewhat less toxic when milk, rather than meat, forms the exclusive diet. Some pathological conditions apparently increase the toxicity of caffein also in dogs. The symptoms of caffein intoxication observed in young dogs are in some respects different from those in full grown and older animals, and resemble those noticed in rabbits.

DISCUSSION OF RESULTS.

It was pointed out at some length in the introduction that the toxicity of some drugs may not be the same for all forms of life. This observation was also made by some investigators who experimented with caffein on different species of animals. Thus Maurel[55] stated that caffein is twice as toxic for the frog as for the rabbit when administered by mouth. Fröhner's[26] experiments, on the other hand, made on domestic animals, failed to show great differences in the toxicity of caffein. According to this observer, horses seem to be more susceptible than cattle, goats, and swine, the minimum toxic dose being the same for all of these, while the resistance of the dog to caffein is about midway between that of the horse and the other animals mentioned. It may be remarked, however, that Fröhner made only 13 experiments. That these data are inadequate for the formation of any conclusions as to the toxicity of caffein is evident since the most striking effect of caffein observed in the work herein reported was the comparatively wide range of variation in the resistance of individuals of the same species to this drug. This was found to be the case even when the conditions of experimentation were approximately uniform, and was observed whatever the mode of administration of the drug employed. The toxicity for different individuals also varied in acute as well as in chronic intoxication. It is for this reason that the number of tests employed were often quite large, for no conclusions of any value could be drawn without averaging the results of a sufficiently large number of experiments. Furthermore, it is to be borne in mind that the action of a drug may differ according to the mode of its introduction into the body and that different species of animals may vary in this regard. This is especially true of some substances when given by mouth, the range in toxicity for certain species of animals being much greater when thus administered than when injected subcutaneously or intravenously.

Maurel's [56] investigations are of interest in this connection, as his work embraces a systematic study of the toxicity of a large number of substances in the rabbit, pigeon, and frog when given by mouth, subcutaneously, intravenously, or when injected into the muscles. According to this investigator the range of variation of the toxicity of a substance is widest when given by mouth. Potassium sulphocyanid, for example, is about 2.5 times as toxic for the frog as for the rabbit when given by mouth. Quinin hydrobromid is three times as toxic for the frog as for the pigeon, while for the rabbit it is twice as toxic as for the pigeon. When given by hypodermic injection the toxic dose per kilo weight is practically the same for all three species. The difference of resistance according to the mode of administration is even more marked for spartein sulphate. When given by mouth the toxicity for the rabbit is six times as great as for the frog, but when injected subcutaneously the toxic dose is about the same for the rabbit and for the frog. The relation of the mode of administration to toxicity is further shown in the following substances: For the rabbit the minimum fatal dose per kilo of quinin hydrobromid is 1.5 grams administered by mouth, 0.5 gram when injected subcutaneously, and 0.07 gram by the intravenous path, while strychnin sulphate is twice as toxic administered intravenously as subcutaneously, and six times as toxic as when administered by mouth. The mode of introduction, however, does not always affect the toxicity of a substance. This is made evident by the action of strychnin on frogs in which, according to Maurel [56], the toxic dose is the same whether given by mouth or injected into the subcutaneous tissues. This appears to hold true also for other animals as demonstrated by the experiments of Hatcher [35] on the cat, in which he observed that strychnin is as readily absorbed from a full stomach as from the subcutaneous tissues. These findings are extremely interesting, especially in view of Maurel's [57] work on the subject, according to which he finds that a substance is much less toxic when given by mouth than when administered by hypodermic injection or intravenously. That this generalization does not admit, however, of universal application is made evident by the work of various experimenters. Claude Bernard [10] observed that curara is as poisonous for the pigeon when given by mouth as when injected subcutaneously, while Zalesky [86] found that samandarin is more toxic for frogs when introduced into the stomach than by injection into the lymph sacs. Our experiments with caffein likewise show that Maurel's generalization does not always hold good, since it was found in experiments with gray rabbits that the minimum fatal dose is but moderately greater by mouth than by the subcutaneous path.

Equally interesting is the observation of the writer, that in the guinea pig the difference in the toxicity between the subcutaneous and intraperitoneal injections is very slight, while in the cat the toxicity of caffein is the same whether given by mouth or injected into the subcutaneous tissues, and is markedly less when injected into the peritoneal cavity. The experiments on dogs show considerable variation of effective dose when given by mouth, but the interesting observation was made that the toxic dose by mouth may be smaller in some cases than the average dose by subcutaneous injection. If the resistance to caffein by subcutaneous injection of the different species of animals experimented upon in the present research be compared, it will be noticed that the gray rabbit or Belgian hare, which is more resistant than the other varieties employed, stands more caffein in proportion to the weight of the body than the other animals.

Although the minimum fatal dose was found to be somewhat larger for the guinea pig than for the gray rabbit when caffein was injected intraperitoneally, it was on the contrary smaller by other paths of introduction, and approximated quite closely the minimum fatal dose for rabbits of the other varieties. Cats as well as dogs were found to be distinctly less resistant to caffein than the herbivora.

There are a number of factors far more important than zoological differences which influence the toxicity of caffein. Some of these are age, season, and pathologic conditions. As these factors have already been dwelt upon in their appropriate places, further discussion might seem unnecessary, but owing to their importance in determining the action of a drug, emphasis is desirable. Especially is this the case with pathological conditions in relation to toxicity. While no positive proof of diminished resistance to caffein in pathological conditions was obtained by subjecting the suggestion to experimental test, it was observed in these experiments on rabbits that death occurred in some individuals after small doses which are usually not even toxic. The findings at autopsy indicate the presence of pathological conditions. The same was observed in some experiments on cats and dogs. It is extremely probable, therefore, that disease modifies the reaction of the organism to caffein as well as to other drugs.[78]

That the resistance to drugs may vary according to the age of the subject has been maintained by a number of pharmacologists. According to Guinard,[80] young dogs, rabbits, and guinea pigs are very susceptible to morphin, resembling children in this regard.[a] The minimum fatal dose for these animals is about one-third less than for the adult. This is not true, however, for the young of other species. Cats under 15 days of age tolerate twice the toxic dose of morphin for the adult cat. Young beeves and goats are likewise more resistant to this alkaloid than adults. On the other hand, according to Livon,[54] young guinea pigs are more sensitive to alkaloids than adults. The toxicity of caffein, as shown in the present investigation, was found to be less in the young than in the adult. In dogs the young subjects are in some instances almost twice as resistant as adults. The difference was found to be less in cats and rabbits than in dogs, but it was quite marked.

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[a] A case of accidental poisoning reported recently by Wichura (München. Med. Woch., 1911, No. 30, p. 1618) throws some doubt on the accepted view that the susceptibility of young children to morphin is greater than that of adults. Wichura also found that the therapeutic doses of codein preparations ordinarily recommended for children in pleuritic cough are not effective in this condition.

The effect of season on the toxicity of drugs has been discussed in the section on the experiments on guinea pigs, which were more resistant to caffein in the fall than in February and March. The effect of season seems to vary with the animal, but it may also differ with the substance employed. In Noe's[65] studies on this subject cantharidin was found to be more toxic for the hedgehog in November than in July. The effect of season was different for morphin, as it was observed that the resistance of the hedgehog was greater at the end of the summer than earlier in the season.

The relation of diet to toxicity of drugs has been studied by Hunt.[39] His experiments indicate that this is an important factor in the resistance to acetonitril. The studies here reported on the effect of diet on toxicity of caffein in rabbits were confined to experiments with oats and carrots and do not show any modification of the resistance to caffein. The question of diet in chronic intoxication in dogs, however, suggests that in these animals diet may affect the toxicity of caffein, although the data on this subject are far from satisfactory. There is nevertheless sufficient evidence to suggest that a high protein diet for the adult dog tends to greater resistance of the animal to caffein and similarly the growing dog tolerates larger quantities of caffein on a milk diet than on a diet of meat.

This brings us to a consideration of the behavior of caffein in chronic intoxication. Although in both rabbits and dogs absence of cumulation was evident, in other respects decided differences in the resistance to caffein were observed. While the rabbit tolerates more than twice the single dose of caffein per kilo for the dog, the result is quite different in repeated dosage of the drug, the rabbit succumbing to continued administration of much smaller doses of the drug than the dog. This is probably due to lesions of the gastro-intestinal canal caused by caffein which occasions loss of appetite much more readily in the rabbit than in the dog. The abundant energy reserve in the dog makes it possible for this animal to stand inanition much longer than the rabbit and other herbivora. The difference in the behavior of the rabbit and dog toward caffein is interesting as showing complete reversal of resistance in acute and chronic intoxication. From the statement in the introduction it is evident that the size of the single toxic or lethal dose of a substance is in no wise an index of the active degree of its toxicity. The experiments with caffein here reported furnish additional evidence that this is true, at least for the rabbit.

GENERAL SUMMARY AND CONCLUSIONS.

The toxicity of caffein in the rabbit varies with the mode of its administration, being least when given by mouth and greatest by intravenous administration. The toxicity is from 15 to 20 per cent greater by subcutaneous injections than by mouth, but is about half of that when injected into the peritoneal cavity. No difference was observed in the toxicity of caffein whether administered into gluteal or into the lumbar muscles. When introduced by this route the toxicity was found to be less by one-third than when it is injected into the peritoneal cavity, but is about 30 per cent more toxic than the subcutaneous injections. White or black rabbits were found to be less resistant to caffein than gray rabbits.

The resistance of the guinea pig to caffein, as of the rabbit, is greatest when given by mouth. The minimum fatal dose is less by intraperitoneal injections, but greater than by subcutaneous injections, thus differing from the rabbit in this regard. The adult cat is less resistant than the guinea pig or rabbit to caffein. The minimum lethal dose by mouth is the same as by subcutaneous, and is less than by intraperitoneal, injection. The minimum fatal dose for dogs was found to be the same by mouth as by subcutaneous injection and is almost the same as for the cat. The toxicity of caffein varies in the guinea pig according to season of the year.

Age is likewise a factor in the toxicity of caffein, young animals being more resistant than the full-grown and older animals; this was shown in experiments on rabbits, cats, and dogs. The symptoms of caffein poisoning also were different in puppies and in full-grown dogs. Different diets, such as carrots and oats, did not influence the resistance of rabbits and guinea pigs to caffein. Low protein diet tends to decrease resistance to caffein in dogs. Young growing dogs are less resistant to caffein on a meat than on a milk diet. Caffein is not cumulative in the rabbit or dog, even if administered for a considerable length of time. Some degree of tolerance may be induced in the rabbit under certain conditions, but not in dogs under the conditions of the experiments made in this investigation. The possibility, however, that dogs may acquire tolerance for caffein is not excluded. Although the rabbit tolerates a much larger single dose of caffein than the dog, it was found, in experiments on chronic intoxication that the rabbit is less resistant to caffein than the dog. The toxicity of caffein is probably increased under pathological conditions, since comparatively smaller doses were fatal to rabbits, cats, and dogs, when marked lesions not due to caffein were found at autopsy. Glycosuria was observed in rabbits, guinea pigs, and cats when caffein was given in sufficient amounts.

TABLE 18.—*Acute caffein intoxication: Table showing average minimum toxic and minimum fatal doses for adult animals.*

Animal.	Effect of dose.	Dose per kilo (grams.)				
		Subcutaneously.	By mouth.	Intraperitoneally.	Intramuscular.	Intravenous.
Rabbit, gray	Toxic	0. 15	0. 325	0. 100-0. 125	0. 13-0. 15	0. 05
	Fatal	.30	.350	.150	.20	0. 10- .16
Rabbit, white or black	Toxic	...	...	...	...	...
	Fatal	.20	.290	...	...	...
Guinea pig	Toxic	0. 15- .16	.150	.200	...	...
	Fatal	.20- .24	0. 280- .300	.240- .250	...	...
Cat	Toxic	.12- .14	.125	.125- .150	...	...
	Fatal	.15	.150	.180- .200	...	...
Dog	Toxic	...	.100- .120	...	...	...
	Fatal	.15- .16	.140- .150	...	...	...

NOTE.—The doses given in this table are approximate.

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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY—BULLETIN No. 149.
H. W. WILEY, Chief of Bureau.

THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS.

BY

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Laboratory of Plant Physiological Chemistry.

WASHINGTON:
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1912.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., June 12, 1911.

Sir: I have the honor to submit for your approval a manuscript prepared by J. F. Breazeale and J. A. Le Clerc, plant physiological chemists, of this bureau, on the growth of wheat seedlings as affected by acid and alkaline conditions. Although the results herein recorded were obtained from experiments conducted in water cultures, it is believed that their interpretation may be directly applied to practical agriculture. I recommend the publication of this manuscript as Bulletin No. 149 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS.

INTRODUCTION.

The following physiological study on the effect of the reaction of the culture medium on the growth of wheat seedlings and particularly on the development of the root was undertaken primarily to determine whether or not the results obtained in practical agriculture can be explained in the laboratory from a purely scientific standpoint.

A few years ago Director Thorne, of the Ohio experiment station, called the attention of one of the authors to peculiar conditions existing on certain of his fertilizer experiment plots, to which potassium chlorid and potassium sulphate had been applied annually for the past 12 years. These plots had become so acid that it was found impossible to grow clover on them. Liming one-half of each plot had restored that half to almost its original fertility. This acidity, he said, was due to the selective action of the root, doubtless a correct explanation which will be considered in detail later.

There seems to be a natural tendency for all soils to become acid under continuous culture, due chiefly to one of two processes. The primary cause of this acidity is the decay of organic matter, leaves, stems, etc., which during decomposition develop acid-reacting bodies. If this process is allowed to continue, the reaction will ultimately become alkaline, but under soil conditions, especially if the aeration is poor and the water level high, decay is apt to be checked, leaving the soil in a characteristic acid condition. This phase of soil acidity, which will not be considered in this bulletin, has been studied extensively during the past few years, particularly by the Rhode Island and Ohio experiment stations, and by Mr. Frederick V. Coville[1] in his elaborate investigations on the blueberry. Peat bogs furnish an exaggerated case of acidity, due to organic matter. The secondary cause of soil acidity is the subject of the investigation herein reported.

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[1] U. S. Dept. Agr., Bureau of Plant Industry Bul. 193.

EXPERIMENTS WITH NUTRIENT SOLUTIONS AND DISTILLED WATER CULTURES.

DESCRIPTION OF SOLUTIONS AND METHOD OF SOWING.

The following experiments were carried on in nutrient solutions and in distilled water cultures, eliminating the disturbing factors due to the presence of soil grains. For the most part wheat was used on account of the ease in handling the young seedlings. The seed, which was furnished by the Pennsylvania experiment station, was washed with warm water for about six hours and then sprinkled on perforated aluminum disks about 12 inches in diameter. These disks were floated, by means of glass tubes, in shallow pans which held about 3 liters of water. In this way approximately 1,000 seeds were used in each pan. The preliminary washing removed all of the readily soluble salts, the actual amounts so removed, as has been shown by the authors in previous experiments[1] of this kind, being quite large. The distilled water was prepared especially for this purpose and a small amount of carbon black was placed in each pan.

After some preliminary work a set of 13 pans was started, as follows:

Solution 1. Distilled water.
Solution 2. Distilled water + 0.5 gram of calcium carbonate.
Solution 3. 150 parts per million of sodium nitrate.
Solution 4. 150 parts per million of sodium nitrate + 0.5 gram of calcium carbonate.
Solution 5. 150 parts per million of potassium chlorid.
Solution 6. 150 parts per million of potassium chlorid + 0.5 gram of calcium carbonate.
Solution 7. 150 parts per million of potassium sulphate.
Solution 8. 150 parts per million of potassium sulphate + 0.5 gram of calcium carbonate.
Solution 9. 10 parts per million of hydrochloric acid.
Solution 10. 10 parts per million of hydrochloric acid + 0.5 gram of calcium carbonate.
Solution 11. 10 parts per million of sulphuric acid.
Solution 12. 10 parts per million of sulphuric acid + 0.5 gram of calcium carbonate.
Solution 13. 150 parts per million of sodium nitrate + 75 parts per million of potassium sulphate.

FIRST CROP OF SEEDLINGS.

These cultures were started January 24 and grown until February 2. The plants were then harvested, representative samples were selected and photographed, and the others were dried, weighed, and prepared for analysis. The photographs are shown in Plates I and II, the controls being the same in each case. The solutions were then made up to volume, 500 cc withdrawn for analysis, and the remainder saved in which to grow a second crop.

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[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 138.

Reaction of the residual solution.—In Table 1 are shown the reactions and titration data for the 13 solutions after gathering the first crop; no nitrates and only a trace of potash remained in any of the solutions.

TABLE 1.—*Titration of the culture solutions after the first crop was harvested.*

No. of solution.	Original solution.[1]	Reaction upon boiling.	Titration of 100 cc with twentieth-normal sodium hydrate.
			cc.
1	Distilled water	Slightly acid	0.1
2	Distilled water+calcium carbonate	Akaline	
3	Sodium nitrate	do	
4	Sodium nitrate+calcium carbonate	do	
5	Potassium chlorid	Acid	.8
6	Potassium chlorid+calcium carbonate	Alkaline	
7	Potassium sulphate	Acid	1.2
8	Potassium sulphate+calcium carbonate	Alkaline	
9	Hydrochloric acid	Acid	.1
10	Hydrochloric acid+calcium carbonate	Alkaline	
11	Sulphuric acid	Acid	.2
12	Sulphuric acid+calcium carbonate	Alkaline	
13	Sodium nitrate+potassium sulphate	do	

[1] See page 6 for amounts of constituents in each solution.

Effects of sodium nitrate.—In Plate I, figure *a*, a slight improvement, especially in root development, is noticed in the distilled-water cultures when calcium carbonate is added, but no appreciable difference is seen when the calcium carbonate is added to the sodium nitrate solution. By reference to the analysis of the culture solutions in Table 1, it will be seen that control No. 1, at the close of the experiment, showed, upon boiling, a slight acid reaction. This was probably due either to the exudation by the roots of some acid-reacting body or to the excretion of similar bodies by the seeds themselves. Nos. 2 and 4 were, of course, alkaline because of the presence of the calcium carbonate. The alkalinity of No. 3 was due to the fact that all of the nitrate (NO_3) or acid radical in the sodium nitrate had been removed by the plant, while some of the sodium or alkaline radical, being less rapidly absorbed, remained in the solution, forming, probably, sodium hydroxid. This, in turn, had taken up carbon dioxid and was existing in the solution as sodium bicarbonate.

Relative effects of potassium chlorid and hydrochloric acid.—In Plate I, figure *b*, are shown the relative effects of potassium chlorid and hydrochloric acid (solutions 5 and 6, and 9 and 10). The plants removed practically all of the potash from the potassium chlorid solution, only a trace remaining, while by analysis 38 parts per million of chlorin out of a total of 71 still remained in the solution, thus clearly showing the selective ability which roots possess to take up one part or radical of a salt and to leave the other. All of this chlorin did not exist as hydrochloric acid, for after boiling and titrating with twentieth-normal sodium hydrate, using phenolphthalein as an indicator, a reading equivalent to only 14.6 parts per million of hydrochloric acid was obtained, which, however, is a much larger amount of free acid than was originally present in solutions 9 and 10, where only 10 parts per million of acid were used.

The effects of the strongly acid solutions were markedly shown upon the roots, that is, in the potassium-chlorid solution they showed the same general characteristics as those grown in the hydrochloric acid; they were short, stubby, and often discolored, the tips being enlarged, and many of the roots growing in the shape of a fishhook, a characteristic of roots grown in the presence of some toxic substance. Moore, Roaf, and Knowles,[1] as a result of their investigation, found that the smallest amount of free acid arrests the growth and nuclear divisions at the root tip, causing it to become thicker, while dilute alkali stimulates the tip to excessive nuclear division.

Of the 10 parts per million of hydrochloric acid added to solution 9, only 1.8 parts remained in solution after the first cropping. The remainder was either absorbed by the plants or the carbon black, or was neutralized by the aluminum disk. Both in the case of the potassium chlorid and of the hydrochloric acid, the presence of calcium carbonate overcame the injurious effects of the acids in the solutions. That is, of course, a common observation and hardly requires citations from the literature to substantiate it. Claudel and Crochetelle[2] found that when they used ammonium sulphate, potassium chlorid, or potassium sulphate in amounts varying from 500 to 5,000 parts per million, an injurious action on germination was noted, and that the application of lime neutralized this toxic effect.

The results obtained by the use of lime in the experiments here reported can be explained only by assuming that the lime has been used to neutralize the acids.

Effect of reaction of the solution on weight of roots and tops.—In making the culture medium alkaline, the growth of the root has been most favorably affected. From Table 2 it is seen that in every case where lime was present the weight of the roots was greater than in the corresponding case without lime, and especially was this so where hydrochloric acid and sulphuric acid had been used, with and without lime.

---

[1] Effects of Variations in Inorganic Salts and the Reactivity of the External Medium upon the Nutrition, Growth, and Cell Division in Plants and Animals. Biochem. J., 1908, *3:* 279.

[2] Les engrais et la germination. Ann. Agron., 1896, *22:* 131

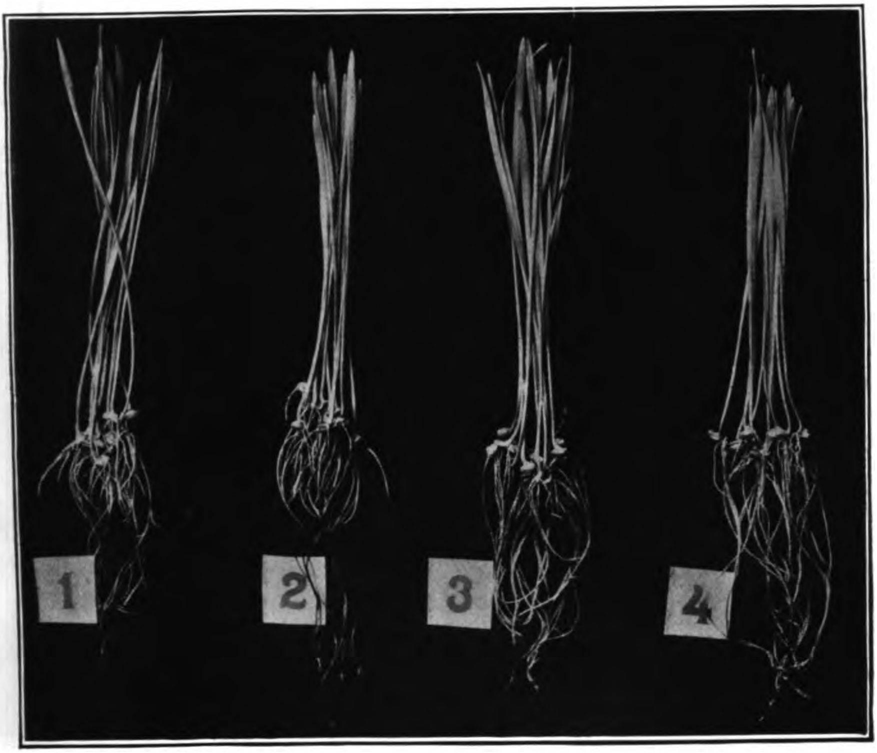

Fig. a.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sodium nitrate; (4) sodium nitrate + calcium carbonate.

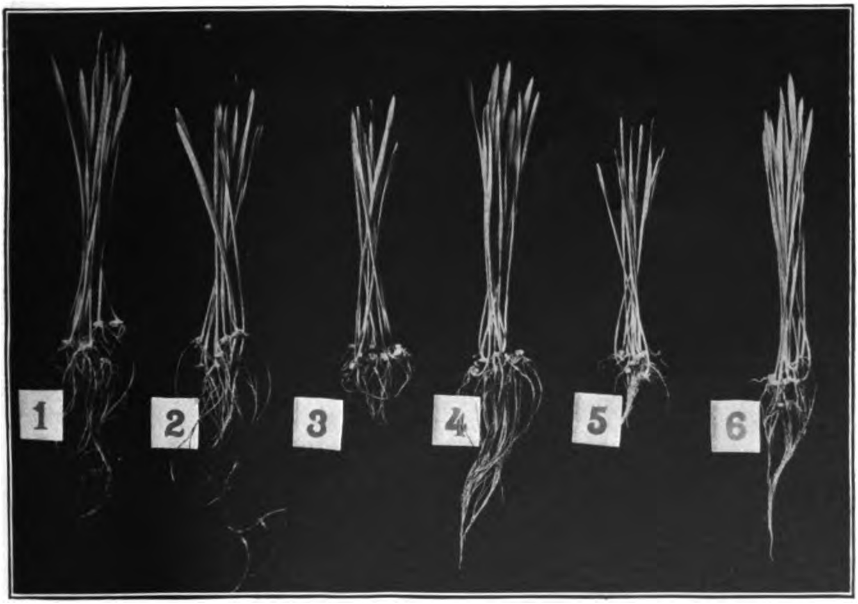

Fig. b.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid + calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid + calcium carbonate.

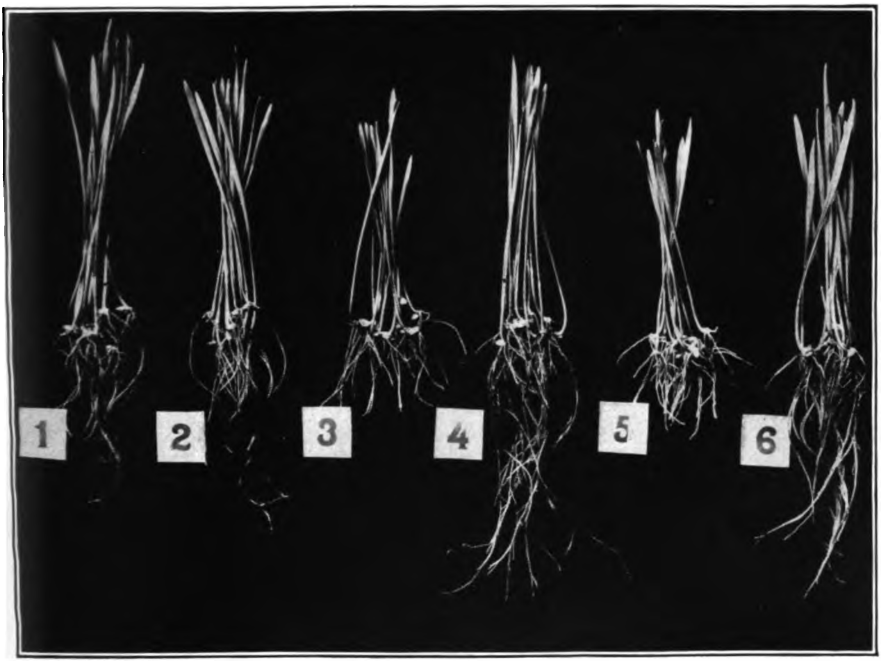

Fig. a.—First crop of plants, grown 9 days in (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate + calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid + calcium carbonate.

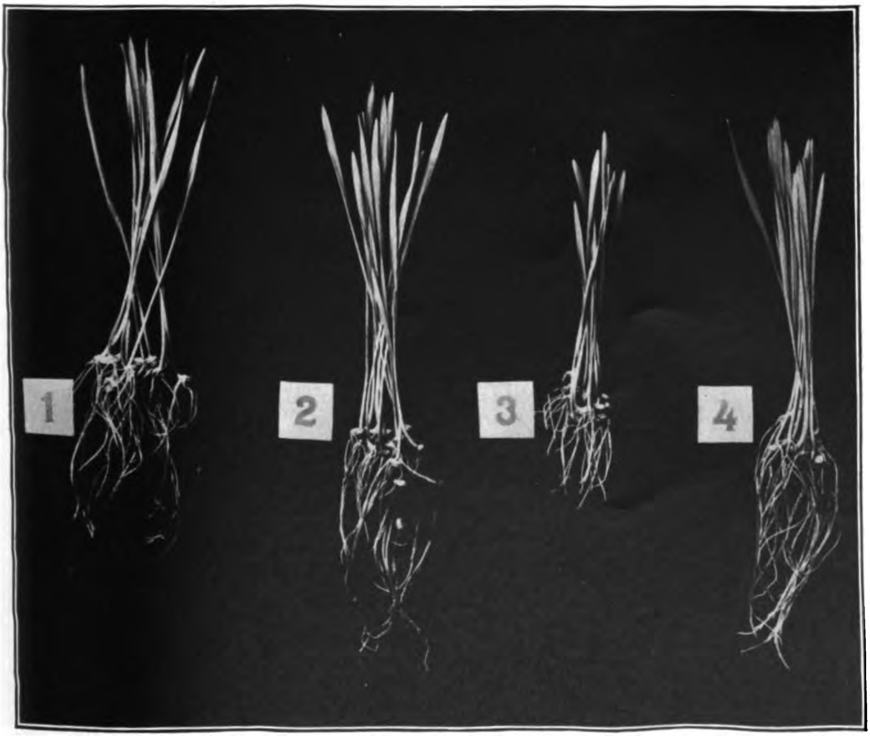

Fig. b.—First crop of plants, grown 9 days in (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate + potassium sulphate.

TABLE 2.—*Dry weight of first crop of plants.*

No. of solution.	Original solution.	100 tops.	100 roots.	100 whole plants.
		Grams.	*Grams.*	*Grams.*
1	Distilled water	1.013	0.575	2.041
2	Distilled water+calcium carbonate	.953	.704	2.176
3	Sodium nitrate	1.012	.625	2.090
4	Sodium nitrate+calcium carbonate	.932	.673	2.135
5	Potassium chlorid	1.088	.466	2.213
6	Potassium chlorid+calcium carbonate	1.013	.639	2.247
7	Potassium sulphate	1.055	.457	2.342
8	Potassium sulphate+calcium carbonate	.933	.672	2.288
9	Hydrochloric acid	.870	.320	2.103
10	Hydrochloric acid+calcium carbonate	.888	.577	2.185
11	Sulphuric acid	.914	.392	1.910
12	Sulphuric acid+calcium carbonate	1.063	.682	2.092
13	Sodium nitrate+potassium sulphate	.878	.528	2.055

The following tabulation shows the increase in weight of one hundred roots in each case when lime was added:

Solution.	Gram.	Solution.	Gram.
Distilled water	0.129	Potassium sulphate	0.215
Sodium nitrate	.048	Hydrochloric acid	.257
Potassium chlorid	.173	Sulphuric acid	.290

These figures indicate that the least increase was found in the sodium nitrate solution. This is explained by Table 1, which shows that even when sodium nitrate was used alone the solution was alkaline at the end of the experiment, and therefore the further use of calcium carbonate simply acted as a stimulant to a very slight extent.

The weight of the tops, or rather of the stems grown in solutions with and without lime, show no such marked difference, indicating that the appearance of the stems of young wheat seedlings is not always a criterion of the vigor of the plant and therefore of the probable yield of the mature crop. In other words, the root system may be a better indicator as to the probable success of a crop than the appearance of the portion above ground.

The effects on the root development, due to the acidity, are always easily noticeable in water-culture experiments such as these, but in field tests the results of applications of single fertilizers, for example, potassium chlorid, potassium sulphate, ammonium chlorid, or ammonium sulphate, are not necessarily apparent in so short a time, due to the fact that there may be many substances in soils which neutralize the acid thus formed.

Relative effects of potassium sulphate and sulphuric acid.—In Plate II, figure *a*, are shown in a similar way the comparative effects of potassium sulphate and sulphuric acid. With the potassium sulphate the plants removed all of the potash, but left 57 out of a total of 82 parts per million of sulphate in solution. This solution showed on analysis 29.4 parts per million of sulphuric acid, an amount more than twice as large as that originally present in solution 11, thus again showing the extent to which the selective action of roots for one radical of a salt may be carried. Leclerc du Sablon[1] put forth the theory that such useful ions as potassium (K), phosphorus (PO_4), nitrogen (NO_3), and iodin (I) combine with the organic substance of the plant, thus relieving the osmotic pressure within the plant and permitting a still larger quantity of these ions to be absorbed. On the other hand, such ions as chlorin (Cl) and sodium (Na) do not form this organic combination, the result being that the pressure inside the plant soon equals that outside, and the plant is, therefore, satisfied with a relatively small amount of them.

Of the 10 parts per million of sulphuric acid present in solution 11, all but 4.9 parts per million disappeared. The appearance of the plant roots in the potassium sulphate solution was even worse than that of those grown in potassium chlorid. Maze[2] obtained results which may be similarly interpreted in his experiments in growing corn in water cultures. He found that when ammonium sulphate was used the crop was not so well developed as when ammonium chlorid was applied. Calcium carbonate overcame the injurious effects in these as in the preceding cultures, thus again showing how beneficial it is to the crop to maintain the alkalinity of the culture medium.

The effect of sodium nitrate and potassium sulphate separately and in combination.—In Plate II, figure *b*, are shown the cultures of sodium nitrate (alkaline reacting), potassium sulphate (acid reacting), and a mixture of the two. The mixture was prepared so as to become alkaline and produce good plants, and it did so. In this case the nitrate of the sodium nitrate and the potassium of the potassium sulphate were drawn up into the plant, while the sodium left in solution from the sodium nitrate combined with the sulphate from the potassium sulphate to form sodium sulphate, with a sufficient excess of sodium to make the solution alkaline.

Discussion of data obtained on the first crop.—All of the culture solutions to which no sulphate was added; that is, the sodium nitrate, the hydrochloric acid, and the distilled water cultures, contained a small amount of SO_4, due probably to its excretion from the seed. In solutions 7 and 8, however, containing 150 parts per million of potassium sulphate, the amounts of SO_4 were noticeably large, 57 out of the 87 parts per million being found in the solution. This was about 6 times more than was found in the sulphate-free solutions and would seem to indicate that the plant at this stage does not require much more than 25 to 30 parts per million of SO_4, since a much larger amount was at their disposition during the whole period without any larger amount being absorbed. These two solutions, Nos. 7 and 8, containing 87 parts per million of SO_4 and 63 parts per million of potash, respectively, show very clearly to what extent selective action is practiced by the roots. At the end of the experiment all of the potash had been taken up by the plant, while only 30 parts per million of SO_4 were thus absorbed.

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[1] Dégagement d'eau par les plantes, Rev. gén. Bot., 1909, *21:* 295.

[2] Compt. rend., 1911, *152:* 783.

Solutions 5 and 6 show the same selective process going on in the case of potassium chlorid. These solutions contained at the beginning of the experiment 79 parts per million of the potash ion and 71 parts per million of the chlorid ion. At the end of the experiment no potash was found in the solution, indicating that the plant had absorbed the whole of this element, while 35 to 38 parts per million of chlorid were still present. These results are at variance with those obtained by J. de Rufz de Lavison,[1] who, after growing the young seedling five days in twentieth-normal potassium-chlorid solution, found that the concentration of the solution was about equal to that originally used. The probable reason for such a deduction is that a potassium-chlorid solution of twentieth-normal strength is equivalent to 3,700 parts per million, a solution so strong that even if the plant did exercise a normal selective action of 30 to 50 parts per million, it would be most difficult to detect such a change in a solution of the concentration mentioned.

The pans containing (1) the control, (2) the potassium-sulphate solution, and (3) the potassium sulphate with calcium carbonate, as given in Plate VIII, figure *a*, show that the differences in the development of the roots are marked and that the few plants selected for the plates previously shown were not exceptions to the rule. In every case the plants grown in the presence of lime presented a healthier and more vigorous appearance than those grown in the corresponding solutions without lime.

While the tops of the plants grown in the culture solutions containing calcium carbonate were larger and presented a much better appearance than the others, yet their dry weight was not necessarily greater; on the other hand, the dry weight of the roots was increased in every case by the addition of calcium carbonate.

Although the original seeds and the seedlings all contained sulphur in organic combination, yet no weighable amount of inorganic sulphate was found in the ash of the plants, except in those to which sulphate or sulphuric acid had been added.

In the solutions to which calcium carbonate had been added the carbon dioxid which was given off by the roots combined with the calcium carbonate and brought a relatively high amount of lime into solution as calcium bicarbonate, making the solutions distinctly alkaline.

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[1] Du rôle électif de la racine dans l'absorption des sels. Comp. rend., 1910, *151:* 675.

SECOND CROP OF SEEDLINGS.

Reaction of residual solution and weight of crop.—After the first crop of seedlings was removed from the pans, more seeds that had been thoroughly washed were sprinkled on the aluminum disks and allowed to germinate and grow as a second crop for five days. Samples were then photographed, as shown in Plates III and IV. The solutions were again made up to their original volumes and titrated with the results given in Table 3.

TABLE 3.—*Titration of culture solutions after harvesting the second crop of seedlings.*

No. of solution.	Original treatment.[1]	Reaction upon boiling.	Titration of 100 cc with twentieth-normal sodium hydrate.
			cc.
1	Distilled water	Acid	0.3
2	Distilled water+calcium carbonate	Alkaline	
3	Sodium nitrate	Acid	.7
4	Sodium nitrate+calcium carbonate	Alkaline	
5	Potassium chlorid	Acid	3.3
6	Potassium chlorid+calcium carbonate	Alkaline	
7	Potassium sulphate	Acid	1.5
8	Potassium sulphate+calcium carbonate	Alkaline	
9	Hydrochloric acid	Acid	.5
10	Hydrochloric acid+calcium carbonate	Alkaline	
11	Sulphuric acid	Acid	.6
12	Sulphuric acid+calcium carbonate	Alkaline	
13	Sodium nitrate+potassium sulphate	Acid	.5

[1] For full description of solutions, see p. 6.

The plants were dried and weighed as in the case of the first crop, the results being shown in Table 4.

TABLE 4.—*Weight of second crop of plants.*

No. of solution.	Original treatment.	Green weight of 100 tops.	Dry weight.		
			100 tops.	100 roots.	100 residual seeds.
		Grams.	*Grams.*	*Gram.*	*Gram.*
1	Distilled water	7.04	1.51	0.499	0.500
2	Distilled water+calcium carbonate	9.25	1.86	.770	.298
3	Sodium nitrate	9.00	1.80	.564	.398
4	Sodium nitrate+calcium carbonate	10.62	1.98	.728	.270
5	Potassium chlorid	6,14	1.49	.434	.616
6	Potassium chlorid+calcium carbonate	9.78	1.89	.700	.390
7	Potassium sulphate	5.62	1.40	.446	.772
8	Potassium sulphate+calcium carbonate	9.22	1.94	.698	.332
9	Hydrochloric acid		1.21	.338	.378
10	Hydrochloric acid+calcium carbonate	10.54	2.09	.734	.380
11	Sulphuric acid	7.08	1.50	.518	.362
12	Sulphuric acid+calcium carbonate	9.64	1.62	.652	.314
13	Sodium nitrate+potassium sulphate	9.12	1.76	.574	.376

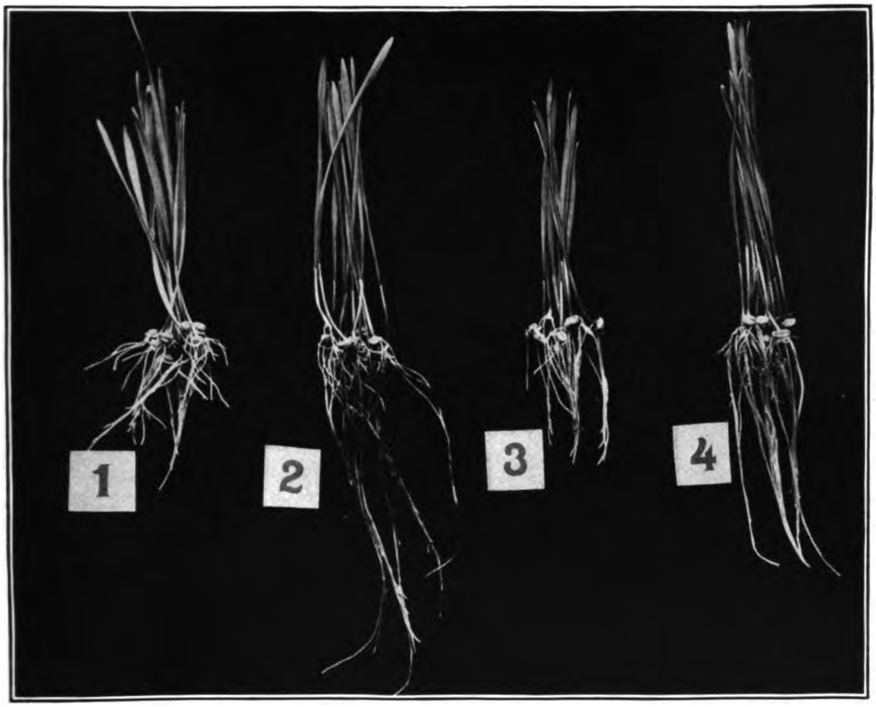

Fig. a.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sodium nitrate; (4) sodium nitrate + calcium carbonate.

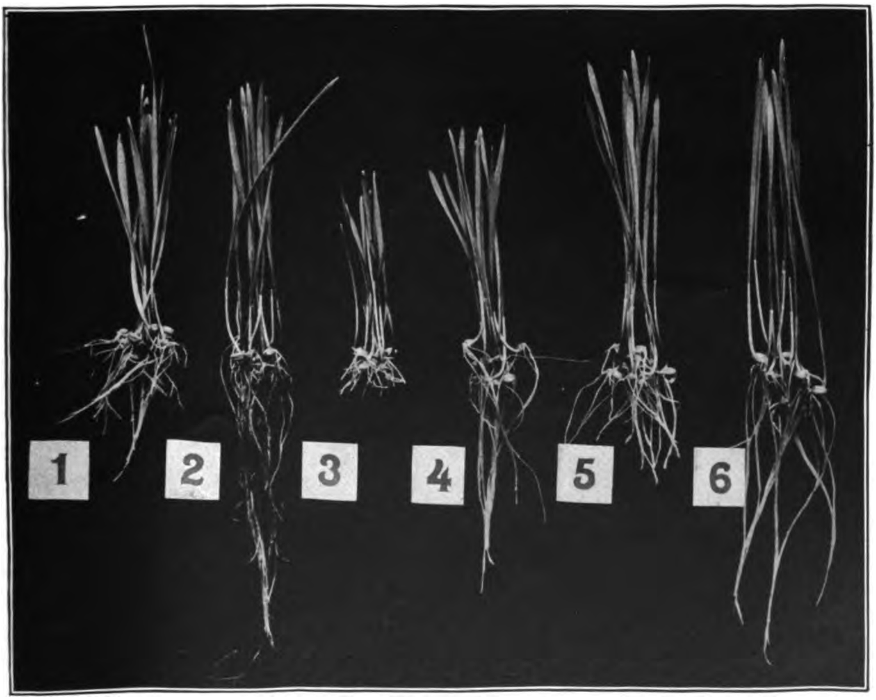

Fig. b.—Second crop, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 5, potassium chlorid; (4) sol. 6, potassium chlorid + calcium carbonate; (5) sol. 9, hydrochloric acid; (6) sol. 10, hydrochloric acid + calcium carbonate.

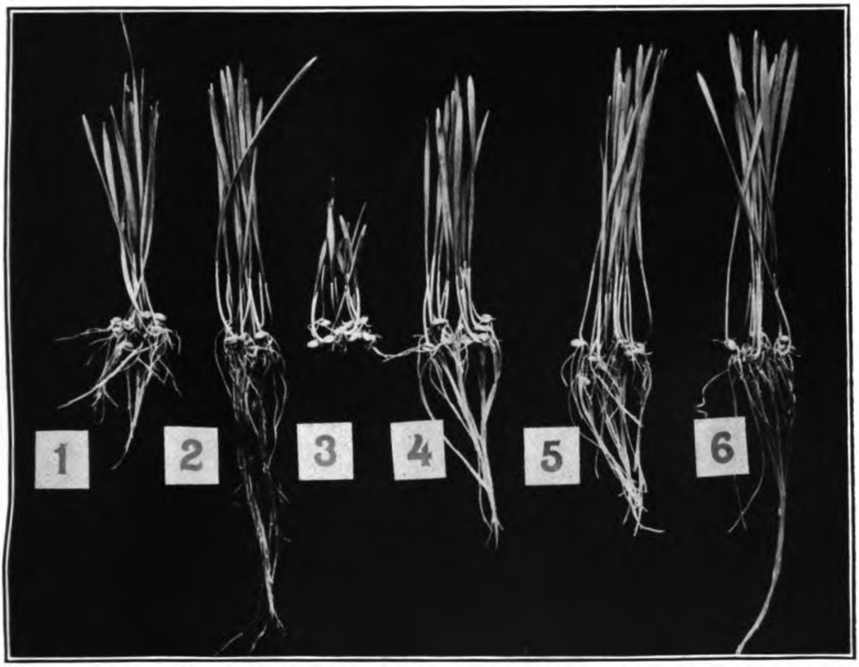

Fig. a.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) distilled water + calcium carbonate; (3) sol. 7, potassium sulphate; (4) sol. 8, potassium sulphate + calcium carbonate; (5) sol. 11, sulphuric acid; (6) sol. 12, sulphuric acid + calcium carbonite.

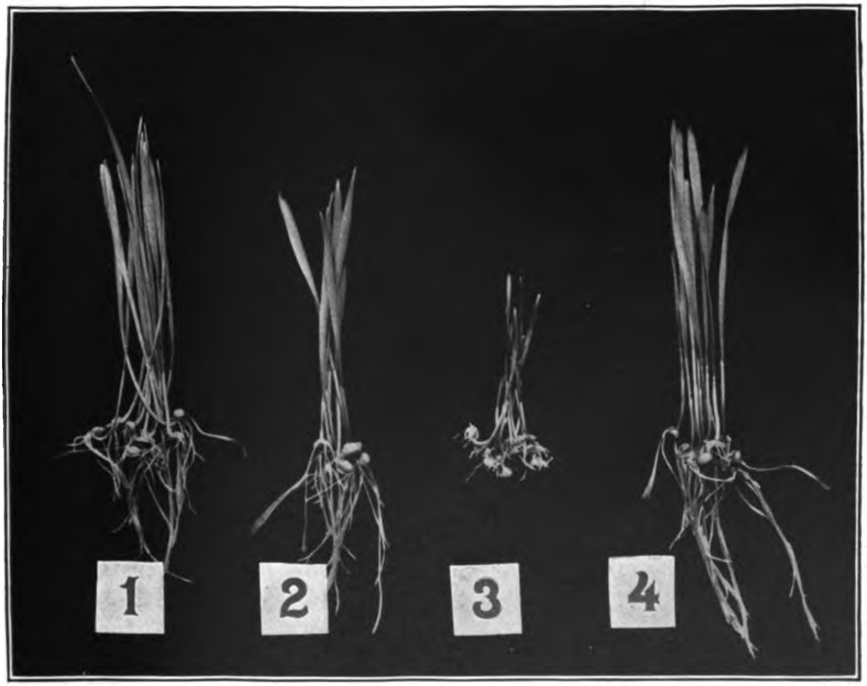

Fig. b.—Second crop of plants, grown 5 days in residual solutions (1) distilled water; (2) sol. 3, sodium nitrate; (3) sol. 7, potassium sulphate; (4) sol. 13, sodium nitrate and potassium sulphate.

Effects of sodium nitrate.—By reference to Plate III, a marked difference will be noted between the distilled water control and the distilled water with calcium carbonate, a difference much greater than was shown in growing the first crop (see Plates I and II). It will also be noticed from Table 3 that the acidity of the solution has increased threefold since the first cropping. Solution 3, Table 3, containing sodium nitrate, which was alkaline after the first cropping, has now also become acid. The sodium evidently either was absorbed by the plant or was neutralized by some organic acid. Solutions 2 and 4, of course, remained alkaline.

Relative effects of potassium chlorid and hydrochloric acid.—In Plate III, figure *b*, the relative effects of potassium chlorid and hydrochloric acid are again shown. The plants grown in the potassium chlorid solution, which, when the second crop was sown, contained no potash but a considerable amount of acid, made practically no root growth and a very poor leaf development. On the other hand, the plants grown in the hydrochloric-acid solution which contained only one-eighth as much acid made a much better growth both in roots and tops.

Relative effects of potassium sulphate, sodium nitrate, and sulphuric acid.—In Plate IV, figure *a*, are shown the residual effects of the potassium sulphate and sulphuric acid. What has just been said of the potassium-chlorid plants and the hydrochloric-acid plants is in the main true of the potassium-sulphate plants and the sulphuric-acid plants also. Figure *b* illustrates the residual effect of sodium nitrate and potassium sulphate used singly and in combination.

COMPARISON OF THE FIRST AND SECOND CROPS.

A striking difference will be noticed between the plants grown in the potash solutions in the first and second crops, when no calcium carbonate was present. The plants in the first crop had practically as much acidity to contend with during the latter days of their growth as the plants of the second crop had during the first few days of their growth, yet the first crop was much better than the second crop. This difference was not due to the fact that the second crop was deficient in potash, for the control pans containing calcium carbonate but no potash gave excellent plants. It is to be noted that the first crop had to contend only with a gradually increasing amount of acidity due to the absorption of potash from the solution in which it was growing, and it was therefore able to accommodate itself and to endure much more acidity than if it had been placed in the stronger acid solution at first, as was done in the case of the second crop. It has been shown by Mr. Jensen, of the Bureau of Plant Industry, in a piece of unpublished work, that the enzymic activity of a wheat seedling is greatest during the first few days of its growth, this being especially true of the oxydases and peroxydases of the root tip. These enzyms are particularly sensitive to acids, and their destruction would mean a cessation of growth. The first crop had evidently passed the critical period with reference to this enzymic activity before the acidity of the solution had developed to such a degree as to prohibit it. When the second crop was placed in the solution the acidity was high enough to practically prohibit the action of the enzyms and the plants were unable to tide over this critical period. The effect of toxic bodies upon the oxydases and peroxydases, that are so active during the first few days of a plant's growth, is an important factor both in agricultural practice and in scientific research and one that is often overlooked.

Many substances that are classified as plant stimulants or depressants no doubt derive their reputation in a great measure from their effect upon the enzyms. Their beneficial or injurious effects would only be noticed during the first few days of the plant's growth when its enzymic activity is so pronounced.

It will be noticed in the case of the second crop that the alkalinity of the solution increased both the green and the dry weight of the tops and the dry weight of the roots. In the case of the residual seeds that were removed from the plants the reverse is true. The presence of an acid in the solution prevented the food material that was stored up in the seed from being transported into the plant. The residual seeds were therefore much lighter when calcium carbonate was present than when it was absent.

After the second crop had been grown in the solutions described for five days the plants were harvested, separated into tops, roots, and residual seeds, and each part dried and weighed separately. The respective solutions were likewise all tested for acidity and the presence of nitrates and potash. Tables 3 and 4 (see page 12) show the results obtained, and a comparison with Tables 1 and 2 (pp. 7 and 9), respectively, will bring out some interesting changes in the residual solution. After the first cropping solution 1 was slightly acid, but after harvesting the second crop it was three times as acid. Solutions 3 and 13 after the first cropping were alkaline, but after the second one both solutions had become quite acid. Analysis of solution 3 showed that while at the beginning there were 40.5 parts per million of sodium (Na), after gathering the second crop only 8 parts per million remained in solution. The difference shows the probable amount of sodium absorbed by the two crops. Not only was the amount thus reduced from 40.5 to 8 parts per million, but the presence of this small amount of sodium seemed to stimulate an acid excretion on the part of the plants to such an extent that the alkaline solution was not only neutralized but was made even more acid than the distilled water culture. On testing for chlorin, after the second cropping, it was shown that only mere traces of that element were found in any of the solutions except Nos. 5 and 6, in which potassium chlorid was used. Even in solutions 9 and 10, to which 10 parts per million of hydrochloric acid were originally added, only traces of chlorin were found. The amounts of chlorin found in solutions 5 and 6 after the second cropping were approximately 5 parts per million on an average, or about one-seventh as much as was present after the first crop was grown. In a similar test for sulphuric acid in the various solutions it was shown that they all contained appreciable amounts, but that solutions 7, 8, and 13, which had been treated with potassium sulphate, contained about two to three times as much sulphuric acid as the others. This acid was apparently either not absorbed to the extent that the chlorin was or else was excreted by the roots in a larger quantity.

The weights of the roots in both crops and of the stems in the second one, when grown with lime, were much greater than when grown without it. The weight of the residual seed, on the other hand, was greater in the unlimed solutions, thus indicating that in an alkaline medium the process of translocation of the seed constituents goes on more readily and completely than under the influence of an acid reaction.

EXPERIMENTS USING ALUMINUM AND FERRIC HYDROXIDS TO REDUCE ACIDITY OF SOLUTION.

A second series of experiments was made, using aluminum hydrate instead of calcium carbonate to keep the solutions from becoming acid. This aluminum hydrate was prepared from aluminum sulphate by precipitation with ammonium hydrate and was washed free from impurities. It was not allowed to dry, but was added as a thick cream. Carbon black was kept in all of the cultures as in the calcium carbonate series. Plate V shows the effect of the aluminum hydrate used instead of lime in the solutions containing potassium chlorid, hydrochloric acid, potassium sulphate, and sulphuric acid. The results after the plant had grown five days indicate that many substances, which are capable of neutralizing the acid of the culture medium and of keeping it alkaline, may be of benefit to the seedling.

In Plate VI, figure *a*, is shown the relative effect of the addition of aluminum hydrate to solutions containing 150 parts per million of sodium nitrate and 10 parts per million of sodium hydrate. There is no difference between the two crops, and the aluminum hydrate failed to produce any effect upon either. This indicates that, at this stage of growth at least, the reaction of the solution is of more importance than the added plant foods.

Plants were withdrawn from these pans and arranged as in figure *b* to show the effect of the potassium nitrate. This salt gave an alkaline reaction to the solution and produced excellent plants. The "fishhooks" on the roots of the potassium sulphate plants are seen in this plate also (No. 4).

The experiment with aluminum hydrate was repeated with ferric hydrate. This was prepared from ferric chlorid by precipitation with ammonium hydrate and was also washed free from all impurities in order to insure freedom from chlorin and ammonium. A blank culture solution prepared with the ferric hydrate contained less than 0.5 part per million of ammonium. The 5-day-old plants are shown in Plate VII, and when compared with the preceding plates show that the same general characteristics prevail whether calcium carbonate, aluminum hydrate, or ferric hydrate is used to keep down the acidity.

EXPERIMENTS USING CLOVER AND TIMOTHY WITH WHEAT.

Another experiment was made with clover and timothy, using calcium carbonate to neutralize the acidity. The small seeds were sprinkled upon pieces of bolting cloth of a large mesh, placed on the aluminum disks, and grown with some wheat seedlings. It was found impossible to obtain satisfactory photographs of the timothy plants, but both the timothy and the clover behaved in the same way as did the wheat plants, and were even more sensitive to the acids. A few of the clover plants are shown in Plate VIII, figure *b*.

Both with the wheat and the clover cultures the effect of potassium chlorid was not so harmful as that of potassium sulphate. In nearly every case a much better root development was obtained in the potassium chlorid solutions.

While it is a well-known fact that the presence of certain solids by their absorptive action stimulate the growth of seedlings in water culture, the objection to the presence of a solid (calcium carbonate, ferric hydrate, or aluminum hydrate) in one-half of the cultures is overcome by the fact that another solid, carbon black, existed in all alike, and that this solid has as great an absorptive power as either of the others.

It is also clearly evident that a salt, potassium sulphate for example, is not taken into the plant as such, that is, as the molecule (K_2SO_4), but rather as an ion (K). When the potassium sulphate (K_2SO_4) is applied to the soil as a fertilizer it goes into solution and dissociates—that is, splits apart into two radicals, K and SO_4. The potassium is taken up as the ion, while for the time being the SO_4 radical remains in solution and combines with the water to form

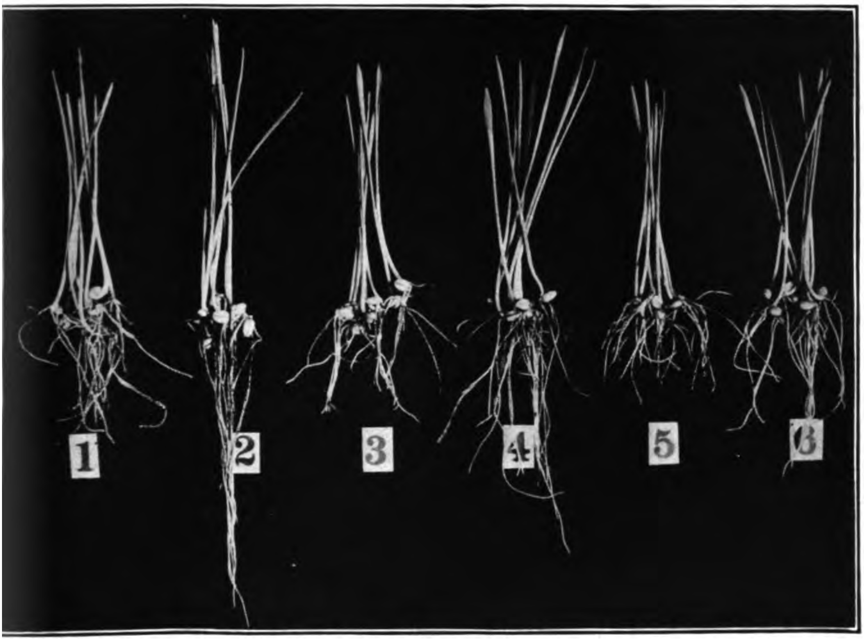

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) potassium chlorid; (4) potassium chlorid + aluminum hydrate; (5) hydrochloric acid; (6) hydrochloric acid + aluminum hydrate.

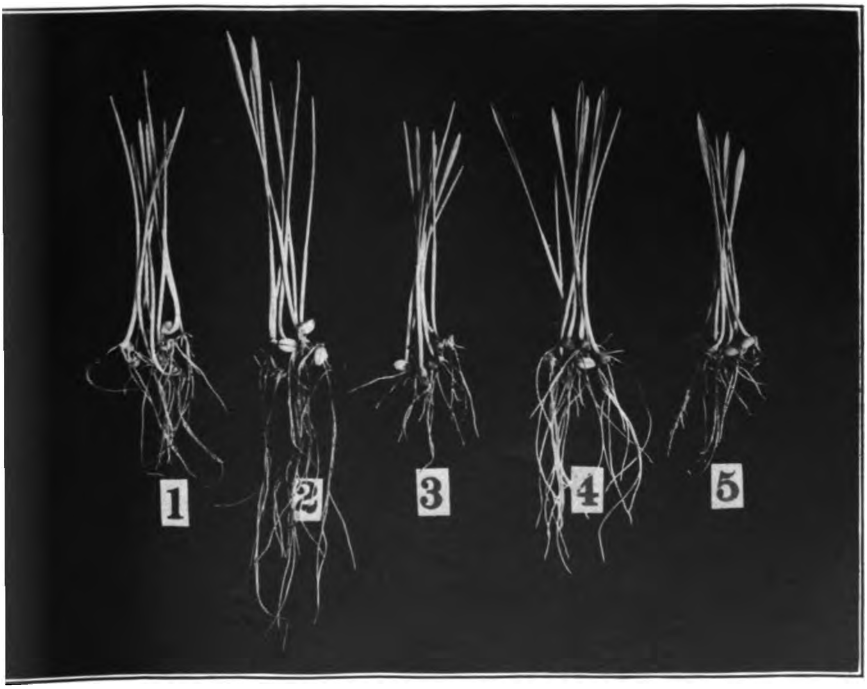

Fig. b.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) potassium sulphate; (4) potassium sulphate + aluminum hydrate; (5) sulphuric acid.

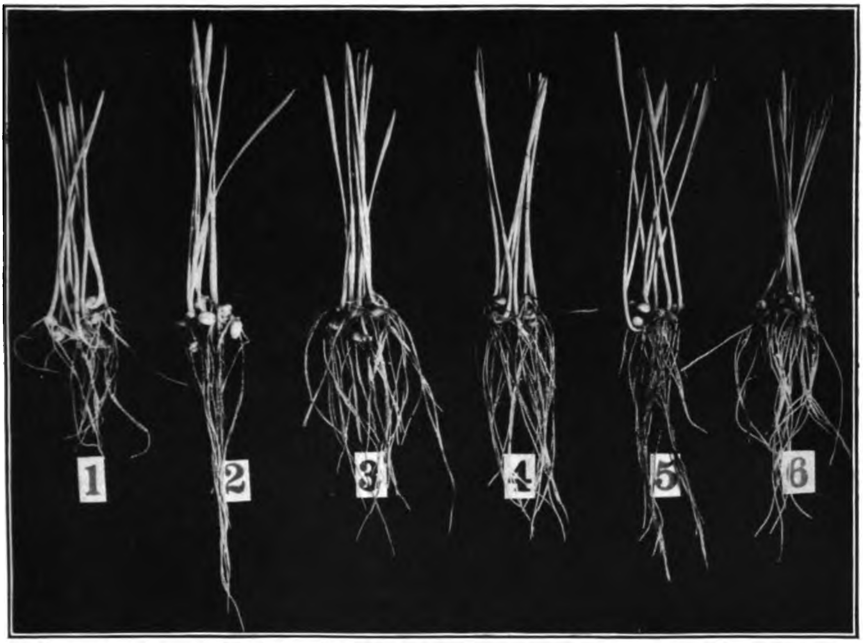

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + aluminum hydrate; (3) sodium nitrate; (4) sodium nitrate + aluminum hydrate; (5) sodium hydrate; (6) sodium hydrate + aluminum hydrate.

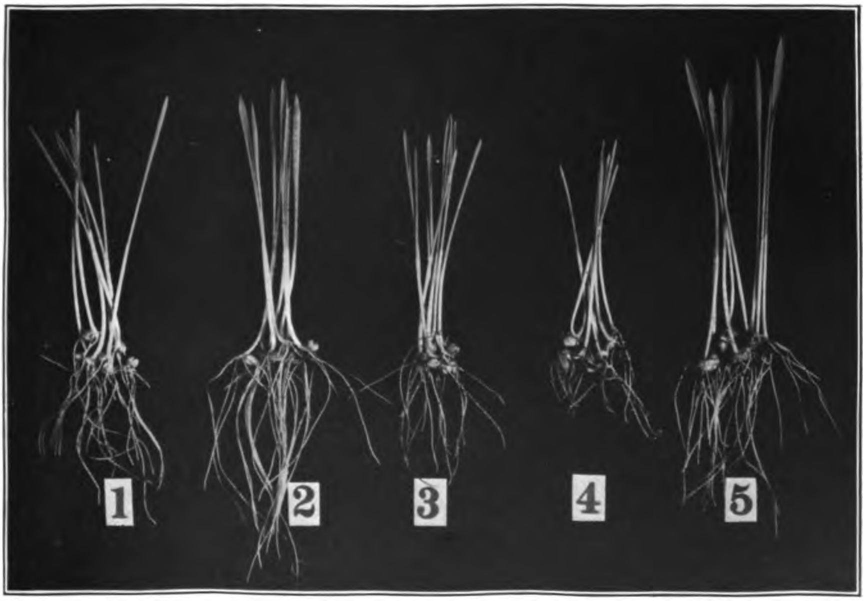

Fig. b.—Crop grown 5 days in (1) distilled water; (2) sodium nitrate; (3) potassium chlorid; (4) potassium sulphate; (5) potassium nitrate.

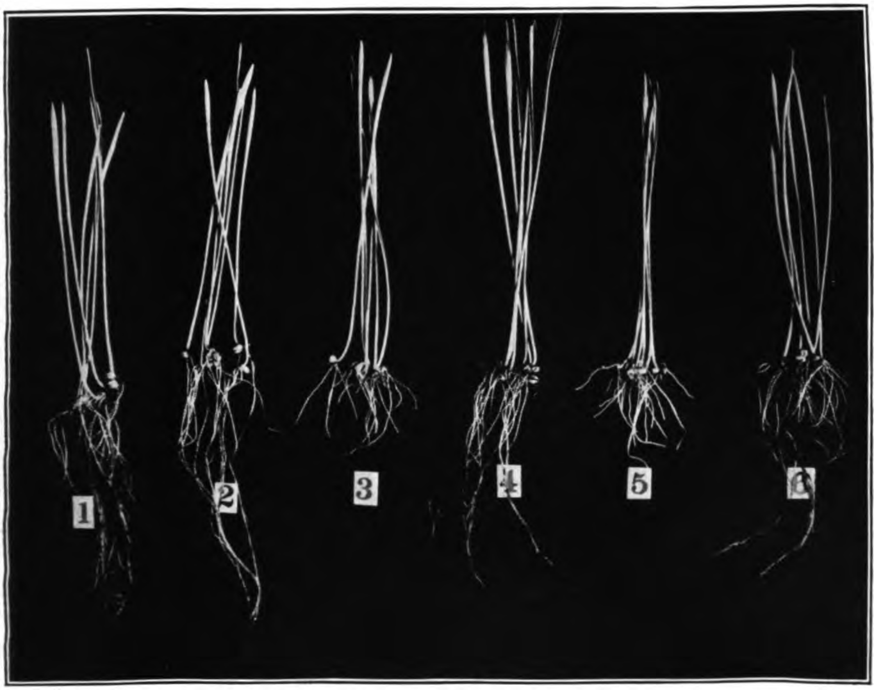

Fig. a.—Crop grown 5 days in (1) distilled water; (2) distilled water + ferric hydrate; (3) potassium chlorid; (4) potassium chlorid + ferric hydrate; (5) hydrochloric acid; (6) hydrochloric acid + ferric hydrate.

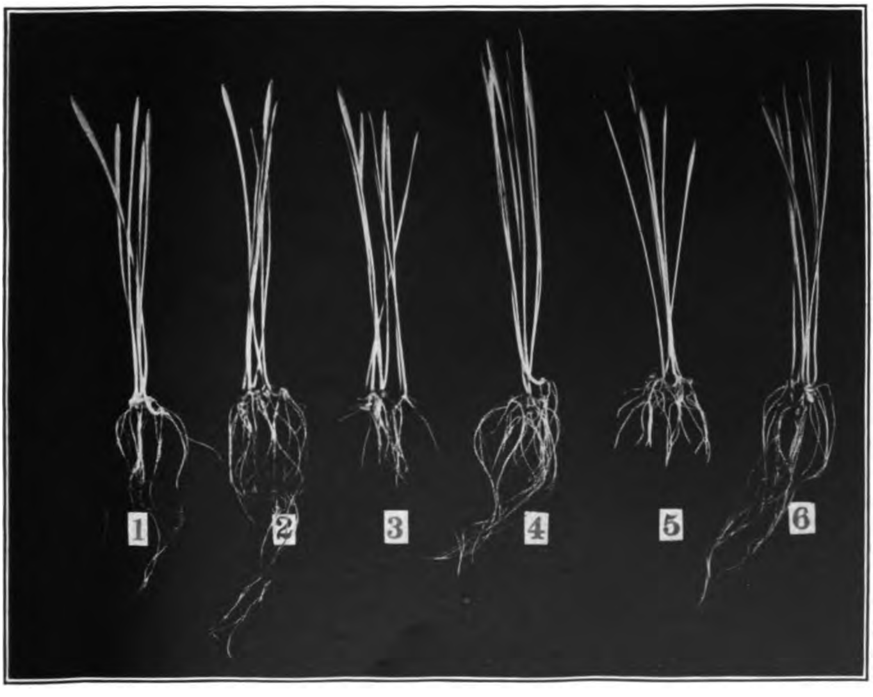

Fig. b.—Crop grown 5 days in (1) distilled water; (2) distilled water + ferric hydrate; (3) potassium sulphate; (4) potassium sulphate + ferric hydrate; (5) sulphuric acid; (6) sulphuric acid + ferric hydrate.

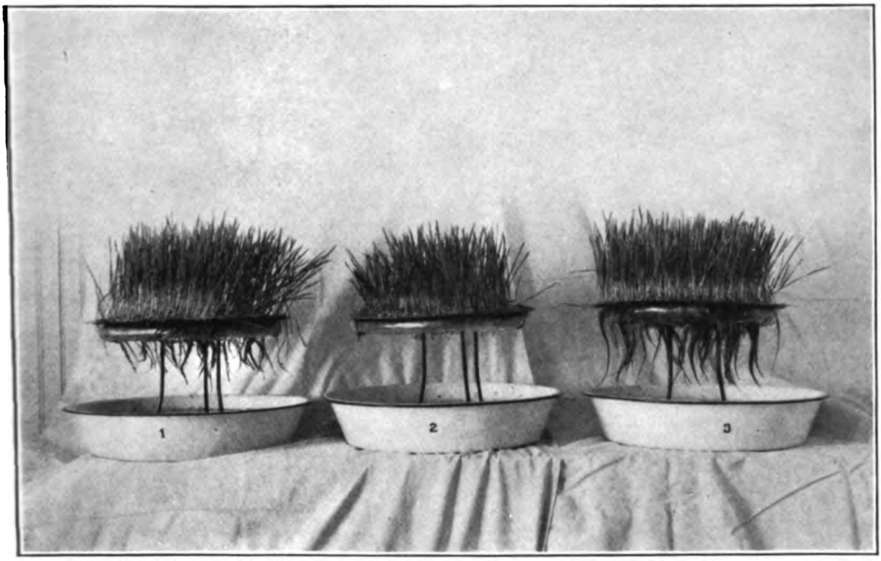

Fig. a.—Comparison of entire pan of wheat plants grown in (1) distilled water; (2) potassium sulphate; and (3) potassium sulphate + calcium carbonate.

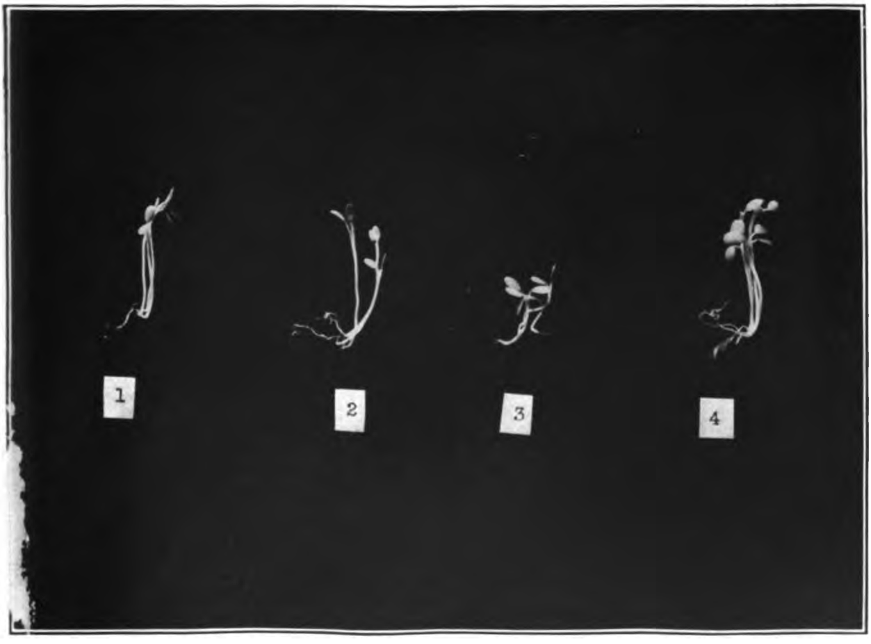

Fig. b.—Clover plants grown with wheat in (1) distilled water; (2) distilled water + calcium carbonate; (3) potassium sulphate; (4) potassium sulphate + calcium carbonate.

sulphuric acid.[1] This is a great secondary source of soil acidity and clearly illustrates the faculty of selective absorption by the plant.

CONCLUSIONS.

The agricultural significance of the physiological study described in this bulletin will be evident from the following discussion. When a soil is in good tilth with an optimum moisture content, each grain is surrounded by a film of water. The plant root comes in contact with this film, and from it draws the water and the mineral salts necessary for growth. Expressed in physical terms, this film is in equilibrium with the soil—that is, if the soil grain contains a silicate of potassium, as feldspar, for example, the water composing the film will bring into the solution about eight parts per million of potash. If some of this potash is removed by the plant more will go into solution, and the concentration of the film will remain fairly constant as long as any feldspar remains in the soil grain.

Although a difference of opinion exists as to how the plant foods are brought into solution, whether by the action of water, by carbon dioxid, or by acids which are exuded by the root tips, all agree that it is absolutely necessary for them to be in solution before they can be taken up by the plant. The soil may, therefore, be considered as a storehouse of reserve material, while the film of soil solution may be considered as a plain nutrient solution in which the plant grows. Whether this soil solution is acid or alkaline is one of the most important factors in plant growth, as has been noted in the foregoing physiological study.

The acidity or alkalinity determines to a great extent not only the particular kind of crop to be grown upon the soil, but also the yield of the crop. No one would think of trying to grow alfalfa in a peat bog or cranberries in a limestone soil. On the other hand, a soil which is naturally alkaline will, under continuous cropping with a rotation containing clover, for example, give smaller and smaller yields as the alkalinity is diminished, until it will be found impossible to grow this legume on account of the acidity of the soil. This phenomenon has been often noted in the limestone regions, particularly in the Shenandoah Valley of Virginia, where soils derived from limestone rocks, and even now having the undecomposed limestone within a few feet of the surface, have become so acid that clover can no longer be advantageously grown. Ground limestone applied to the surface soil will often restore its original fertility.

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[1] In this connection mention should be made of the experiments of Micheels (Action des liquides anodiques et cathodiques sur la germination. Bull. Acad. Roy. Belg., Classe des Sci., 1910, *5:* 391), who studied the effect on wheat seedlings of an electric current produced by a pile of 24 Daniell cells, when passing through various nutrient solutions; and who found that the plants grown near the anode developed roots whose length varied from 10 to 25 mm, while those plants in the cathode solution possessed roots from 140 to 150 mm in length. Similar results were obtained in nutrient solutions containing sodium chlorid, potassium chlorid, potassium nitrate, sodium nitrate, and mixtures of these. A second crop being grown in each of these solutions, even after the electric current was discontinued, similar results were obtained as with the first crop, due to modifications brought about in the solution. From our investigations, the injurious effects noted at the anode, as seen from the length of the roots, could well be attributed to the formation of acid, while near the cathode, the solution being alkaline, the conditions were reversed and therefore favorable for root development.

The heavy clay soils from which bricks are made are always acid, have a high lime requirement, and are usually very unproductive. The oxids of iron exist in such soils in a finely divided colloidal form brought about by an acid condition, and they are usually remedied by an application of lime. The poisonous properties of subsoils also may be due in a large measure to their acid character.

Investigators working with solution cultures and using single salts might do well to take into consideration the reaction of the solutions after the plants have been placed in them. The moment seedlings are placed in a solution of potassium sulphate a rapid absorption of potash begins with a consequent formation of sulphuric acid. This sulphuric acid is a disturbing factor and other substances placed in the solution and kept under observation might owe their apparent beneficial effect to the fact that they simply act as bases, but in themselves have no direct influence upon the plant. In field culture the residual injurious effect of an application of sulphate and chlorid of potash can be overcome by mixing the fertilizer with about twice its weight of lime.

In the experiments here recorded it is shown that the seedlings grown in culture solutions containing potassium chlorid, potassium sulphate, or hydrochloric or sulphuric acid solutions (10 parts per million), exert a selective action whereby the potash ion is absorbed by the roots, while the chlorid or sulphate ion is for the most part left in solution. This causes the solution to become acid, which in turn acts injuriously on the root development.

The addition of lime or iron or aluminum hydrate to culture mediums containing potassium chlorid, potassium sulphate, hydrochloric acid, or sulphuric acid, keeps these solutions alkaline so that they then act favorably on the root development. This would tend to explain why field applications of sulphate or muriate of potash in time render the soil acid,[1] and why the continued use of Chile saltpeter produces an alkaline condition of the soil.

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[1] At the Woburn Experiment Station it has been amply shown that a continuous application of ammonium sulphate renders the soil so acid as to cause the crop to be almost an absolute failure. Adjacent plats, likewise treated with ammonium sulphate, but also given an application of lime, have continued to yield crops as large as are produced by still other plats treated with sodium nitrate only.

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